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MAST CELL DEVELOPMENT IN HEALTH AND SYSTEMIC MASTOCYTOSIS

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Cover: illustration of mast cell precursors from the bone marrow (front) and clustering of mast cells in the bone marrow of a mastocytosis patient, similar to figure 6 in this thesis (back).

MAST CELL DEVELOPMENT IN HEALTH AND SYSTEMIC MASTOCYTOSIS

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Listen to advice and accept instruction, and in the end you will be wise

~ Proverbs, the Bible

So I decided there is nothing better than to enjoy food and drink and to find satisfaction in work. Then I realized that these pleasures are from the hand of God

~ Ecclesiastics, the Bible

ABSTRACT

Hematopoietic progenitors in the bone marrow differentiate into several immune cells, including mast cells. Mast cell progenitors leave the bone marrow, circulate through the blood, and home to the periphery where they mature. This thesis aims to gain more insights into the development of mast cells in health and in the mast cell disease systemic mastocytosis (SM). This disease is characterized by infiltrates of neoplastic mast cells in different tissues and the presence of the D816V mutation in the *KIT* gene. In this thesis we study mast cells and their progenitors in peripheral blood and bone marrow by using multi-color fluorescence-activated cell sorting that enables characterization and isolation of cells of interest. Sorted cells were analyzed using culture experiments, cytochemical stainings techniques, and genetic mutation profiling.

In study I we analyzed peripheral blood samples from SM patients for the presence of mast cells, which are absent in blood of healthy subjects. In this study we demonstrated the presence of circulating mature mast cells in the peripheral blood of patients with advanced forms of SM.

In study II we assessed the role of SCF and KIT signaling in mast cell development and differentiation. Treatment with the tyrosine kinase inhibitor imatinib, which prevents KIT signaling, did not reduce the percentage of mast cell progenitors in vivo. Using in vitro culture experiments, we further showed that mast cell progenitors from peripheral blood survive, differentiate, and partially mature without SCF and KIT signaling.

In study III we aimed to investigate the *KIT* D816V distribution throughout the hematopoietic landscape. We developed an assay that allows the detection of the *KIT* D816V mutation in single cells sorted from the bone marrow of SM patient. We detected the *KIT* D816V mutation throughout the hematopoietic landscape, from stem cells to the mast cells lineage. However flow cytometry analysis showed that the frequencies of different hematopoietic stem and progenitors were comparable in SM patients and controls. In addition, we demonstrated that mast cells originate from FcεRI$^+$ bone marrow progenitors, and we described aberrant CD45RA expression on mast cells from SM patients.

In study IV we further investigated the cell-forming potential of FcεRI$^+$ common myeloid progenitors (CMPs) in the bone marrow, identified in study III. We found distinct subpopulations within the FcεRI$^+$ CMPs using antibodies against CD203c and integrin β7. Culture experiments showed that CD203c$^+$ FcεRI$^+$ CMPs differentiate into mast cells and basophils, whereas CD203c$^-$ FcεRI$^+$ CMPs form mast cells, basophils, and erythroid cells. Granulocyte-monocyte progenitors, which form neutrophils, did not generate mast cells and basophils, clearly separating the development of different granulocytes.

Taken together, this thesis provides clinical and biological insights into mast cell development in health and systemic mastocytosis.

LIST OF SCIENTIFIC PAPERS

I. Dahlin JS, Ungerstedt JS, **Grootens Jennine**, Sander B, Gülen T, Hägglund H and Nilsson G.
Detection of circulating mast cells in advanced systemic mastocytosis.
Leukemia (2016), vol. 30(9), p1953-1956.

II. Dahlin JS, Ekoff M, **Grootens Jennine**, Löf L, Amini RM, Hagberg H, Ungerstedt JS, Olsson-Strömberg U and Nilsson G.
KIT signaling is dispensable for human mast cell progenitor development.
Blood (2017), vol. 130(16), p1785-1794.

III. **Grootens Jennine**, Ungerstedt JS, Ekoff M, Rönnberg E, Klimkowska M, Amini R, Arock M, Söderlund S, Mattsson M, Nilsson G and Dahlin JS.
Single-cell analysis reveals the *KIT* D816V mutation in haematopoietic stem and progenitor cells in systemic mastocytosis.
EBioMedicine (2019), *article in press*, available online since April 8th

IV. **Grootens Jennine**, Ungerstedt JS, Nilsson G and Dahlin JS.
Report on a basophil and mast cell progenitor with erythroid potential in the human bone Marrow.
Manuscript

Other relevant papers not included in this thesis

I. **Grootens Jennine**, Ungerstedt JS, Nilsson G and Dahlin JS.
Deciphering the differentiation trajectory from hematopoietic stem cells to mast cells.
Blood Advances (2018), vol. 2(17), p2273-81.

II. Lyberg K, Ali HA, **Grootens Jennine**, Kjellander M, Tirfing M, Arock M, Hägglund H, Nilsson G and Ungerstedt JS.
Histone deacetylase inhibitor SAHA mediates mast cell death and epigenetic silencing of constitutively active D816V KIT in systemic mastocytosis.
Oncotarget (2017), vol. 8(6), p9647-59.

CONTENTS

LIST OF ABBREVIATIONS

7-AAD	7-Aminoactinomycin D
ACK	Ammonium chloride potassium
AdvSM	Advanced systemic mastocytosis
AML	Acute myeloid leukemia
ASM	Aggressive systemic mastocytosis
ASXL1	Additional sex combs-like 1
BCR-ABL	Breakpoint cluster region-Abelson proto-oncogene
BMCP	Basophil-mast cell progenitor
BMMC	Bone marrow mast cell
BNKP	B- and NK cell progenitor
CCL	C-C motif chemokine ligand
CD	Cluster of differentiation
CDP	Common dendritic progenitor
CFU	Colony-forming unit
CM	Cutaneous mastocytosis
CML	Chronic myeloid leukemia
CMML	Chronic myelomonocytic leukemia
CMP	Common myeloid progenitor
CR	Complement receptor
CXCR	C-X-C motif chemokine receptor
DAPI	4',6-Diamidino-2-Phenylindole
EB	Elution buffer
EMA	European medicines agency
EMP	Erythoid-myeloid progenitor
EoBP	Eosinophil-basophil progenitor
EPO	Erythropoietin
ERK	Extracellular-signal-regulated kinase
EZH2	Enhancer of zeste homolog 2
FACS	Fluorescent activated cell sorting
FCS	Fetal calf serum
fcs	Flow cytometry standard
FcεRI	Fc epsilon receptor I
FDA	Food and drug Administration
FMO	Fluorescent minus one
GAPDH	Glyceraldehyde 3-phospate dehydrogenase
GIST	Gastrointestinal stromal tumors
GM-CSF	Granulocyte-macrophage colony-stimulating factor
GMP	Granulocyte-monocyte progenitor
HLA	Human leukocyte antigen
HMC-1.2	Human mast cell-line 1.2
HSC	Hematopoietic stem cell
HSPC	Hematopoietic stem and progenitor cells

IgE	Immunoglobulin E
IL	Interleukin
ISM	Indolent systemic mastocytosis
JAK	Janus kinase
LMPP	Lymphoid-primed multipotent progenitor
MAPK	Mitogen-activated protein kinase
MCL	Mast cell leukemia
MCP	Mast cell progenitor
MDS	Myelodysplastic syndrome
MEP	Megakaryocyte-erythroid progenitor
MGG	May-Grünwald Giemsa
MLP	Multilymphoid progenitor
MPN	Myeloproliferative neoplasm
MPP	Multipotent progenitor
mRNA	Messenger ribonucleic acid
mTOR	Mammalian target of rapamycin
N/KRAS	Neuroblastoma/Kirsten rat sarcoma
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
PD-L1	Programmed death-ligand 1
PI3K	Phosphoinositide-3-kinase
qPCR	Quantitate PCR
RUNX1	Runt-related transcription factor 1
SCF	Stem cell factor
SM	Systemic mastocytosis
SM-AHN	Systemic mastocytosis with an associated hematological neoplasm
SRSF2	Serine and arginine rich splicing factor 2
SSM	Smoldering systemic mastocytosis
STAT	Signal transducers and activators of transcription
TET2	Tet methylcytosine dioxygenase 2
TKI	Tyrosine kinase inhibitor
TNF	Tumor necrosis factor
VEGF	Vascular endothelial growth factor
WHO	World health organization

1 INTRODUCTION

As the title of this thesis describes, the work of this thesis focuses on the development of mast cell in normal conditions and in the mast cell disease called systemic mastocytosis (SM). The introduction gives an overview of current knowledge on mast cells, mast cell precursors and systemic mastocytosis. This provides the necessary background to understand the findings and discussion of the four studies included this thesis.

1.1 THE IMMUNE SYSTEM

All organisms have a system that protects itself from invaders, the immune system. In this thesis we focus on the mast cell, a tissue-resident immune cell with typical granules first described by Paul Enrich. Evolutionary primitive organisms have a similar granulated cell type that circulates in the hemolymph (figure 1). This cell has granules that contain heparin and histamine, similar to the human mast cell, thus the cells are evolutionary conserved (de Barros et al., 2007). During evolution, organisms became more complex which required a more complex defence system. Most vertebrates have an immune system that consists of a two-layered defence system, a first line defence, the innate immunity and a second line of defence, the adaptive immune system. Humans have more than ten different types of white blood cells, primarily divided into granulocytes, monocytes, and lymphocytes. Some immune cells circulate through the blood and can leave to a tissue where they detect a danger or stranger signal in the human body. Other immune cells reside in tissue where they control the local environment, providing the first line of defence by creating a site of inflammation upon an infection. Other cells in the same area can detect the same antigen or react to the inflammatory reaction of other cells and migrate to lymph nodes where they pass it on to the second line of defence. Cell communication is either direct by receptor-ligand interactions, or indirect by the release of certain factors locally, or at long distance using small vesicles.

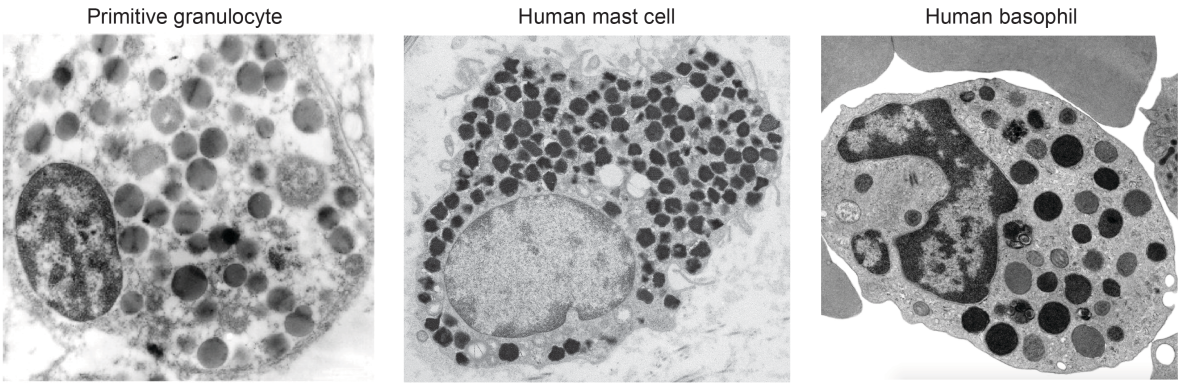

FIGURE 1. Electron microscope images showing electron dense granules in a primitive granulocyte, human tissue-resident mast cell with a round nucleus, and human blood basophil with a bean-shaped nucleus. NB. Sizes are not directly comparable. Adapted and reprinted with permission from the American Society For Biochemistry & Molecular Biology and BioMed Central from de Barros et al. (2007), Friesen et al. (2011) and from Clark Brelje and Sorenson (histologyguide.org).

Together this creates a very complex system with a highly organized communication network. Thus, although this thesis focuses on mast cells, it is important to keep in mind that they are part of this complex network.

1.2 HEMATOPOIESIS

Hematopoiesis describes the development of blood cells, including mast cells. In this thesis we study human hematopoiesis using primary cells and ex vivo culture systems. When studying human diseases, this is the most informative approach because it is more relevant in developing human therapeutics and accounts for species-specific effects as opposed to mouse strains that have a similar genetic background. Nevertheless, in vivo mouse models are useful for studying the effect of genetic loss of function and gain of function alterations related to human oncogenesis and complement human cell models when carried out in parallel.

In adults, all blood cells stem from a common ancestor that resides in a special niche in the bone marrow, the hematopoietic stem cell (HSC). During embryogenesis there is no bone marrow niche, thus in the embryo the first hematopoietic cells arise in the extra-embryonic yolk sac. During further development, the production of hematopoietic cells moves to the aorta-gonad-mesonephros region, the placenta, the fetal liver, the spleen, and eventually to the bone marrow. The bone marrow niche provides the necessary microenvironment that is needed for HSC homeostasis, including quiescence, proliferation, self-renewal and differentiation. Besides other mature hematopoietic cells, the niche contains non-hematopoietic cells such as endothelial cells, adipocytes, supporting mesenchymal stromal cells, and osteoclasts and osteoblast. Local signals from the microenvironment, or from an infection or inflammation can influence and change the HSC homeostasis, which affects the hematopoiesis. A genetic defect in one or multiple cells of the hematopoietic system can also affect the development of hematopoietic cells and cause a myeloid or lymphoid neoplasm (Morrison and Scadden, 2014; Ogawa, 2019; Zon, 2008).

The differentiation of HSCs follows different trajectories in which the classical model separates lymphoid cells from myeloid cells (figure 2). The myeloid cells include all granulocytes, erythrocyte progenitors and megakaryocytes. Surface receptor expression, mRNA expression as well as morphological features identify specific progenitors. Human HSCs as well as all myeloid and lymphoid progenitors express CD34, first identified in 1984 (Civin et al., 1984). Long-term in vitro assays identify HSCs by their capability to produce hematopoietic cells for more than six weeks (Spangrude et al., 1988; Sutherland et al., 1989). Transplantation of potential HSCs into humanized immunodeficient mice models further show their capacity to form all different blood cells, revealing their multipotency and long-term self-renewal capacity (Shultz et al., 2005). Perhaps even better proof that $CD34^+$ cells can replenish a whole body with blood cells comes from human allogeneic HSC transplants for the treatment of lymphoid and myeloid leukemia.

Human HSCs express CD90 (Thy1) and CD49f (Majeti et al., 2007; Notta et al., 2011). CD90 and CD49f separate HSCs from the multipotent progenitor (MPP), both lacking expression of

CD38 and CD45RA. This change in expression to $CD90^-$ and $CD49f^-$ MPPs represents the earliest steps of HSC differentiation.

Further differentiation describes the segregation into a common myeloid and common lymphoid progenitor. The common myeloid progenitor (CMP), branches down into megakaryocyte-erythroid progenitors (MEPs) and granulocyte-monocyte progenitors (GMPs) (Manz et al., 2002). These cell progenitor populations express CD38 and have differential expression of CD123, and CD45RA (Manz et al., 2002).

Later studies showed that not all myeloid cells develop through a common myeloid progenitor (Görgens et al., 2013). Lymphocytes, monocytes and neutrophils differentiate though the lymphoid-myeloid primed progenitor (LMPP) (Goardon et al., 2011) (figure 3; hierarchical model). Megakaryocytes, erythrocytes, and other myeloid cells such as basophils and eosinophils differentiate through the common erythroid-myeloid progenitor (EMP). LMPPs give rise to lymphocytes and NK cells through the multi-lymphoid progenitor (MLP) (Doulatov et al., 2010). In the LMPP-EMP model, the GMP gives monocytes and neutrophils, but not other granulocytes, which is important to remember when using the nomenclature "GMP". Furthermore, this model also suggests the existence of an eosinophil-basophil progenitor (EoBP), as shown before (Denburg et al., 1985). Eosinophils and basophils grow in vitro cultures supplemented with IL-3 and IL-5 (Boyce et al., 1995). The IL-5 receptor (IL-5R/CD125) is a marker for eosinophils and eosinophil progenitors (Mori et al., 2009). Similarly, IL-3 binds to the IL-3 receptor (IL-3R/CD123), which is the main growth factor for basophils (Valent et al., 1989). In addition, CD34 and CD203c are present on basophil progenitors, but cultured $CD34^+$ $CD203c^+$ cells do not exclusively give rise to basophils (Buhring et al., 1999). Thus, there are some potential markers that can identify basophil progenitors, but a unipotent basophil progenitor is still to be isolated.

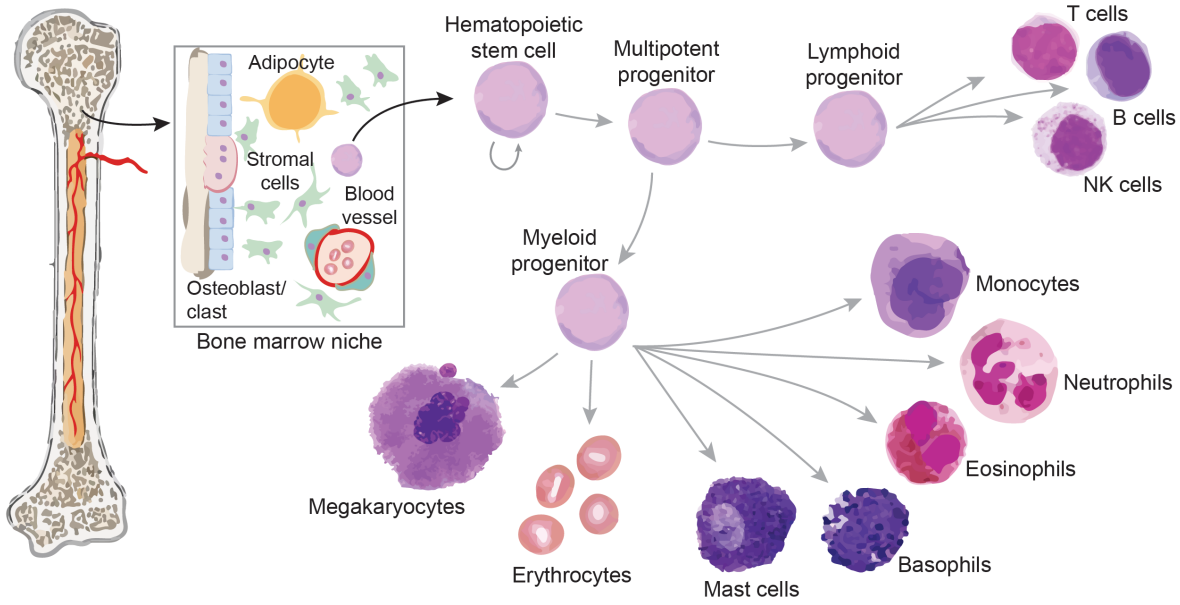

FIGURE 2. Human adult hematopoietic stem cells reside in a special niche in the bone marrow and differentiate into lymphoid and myeloid cells.

The LMPP-EMP model does not describe mast cells, which circulate in the blood as mast cell progenitors (MCPs) (Dahlin et al., 2016). However mast cells likely develop from EMPs, rather than LMPPs because of their similarity to basophils, also packed with electron-dense granules (figure 1). Mast cells and basophils both express the high-affinity IgE receptor, FcεRI, whereas expression of the receptor for stem cell factor (SCF), KIT (CD117) distinguishes mast cells from basophils. Hematopoietic cells express KIT throughout development and mast cells upregulate KIT when they mature, whereas basophils and most other blood cells lose KIT expression (Irani et al., 1992). Whereas eosinophils, basophils, and mast cells have some phenotypical similarities, transcriptional data clearly shows that mast cells, basophils, and eosinophils present a unique transcriptional signature (Dwyer et al., 2016; Motakis et al., 2014).

The application of single-cell sequencing techniques in hematopoiesis research changes the view on the process of blood cell development further, questioning the existence of a hierarchical model of hematopoiesis. It is becoming increasingly clear that hematopoiesis is a gradual process without stable intermediate progenitors (figure 3; gradual model) (Notta et al., 2016; Paul et al., 2015; Tusi et al., 2018; Velten et al., 2017; Zheng et al., 2018). In the gradual model for hematopoiesis, the phenotypic progenitors from the classical models do not constitute discrete progenitor cells types, but rather a transitory state. This means that different cell lineage can emerge directly from the HSC, without passing through separate, stable progenitors. However in this model, intermediate progenitors can still be identified which describe differentiating progenitors that have a restricted potential.

1.3 MAST CELL DIFFERENTIATION

Mast cells develop from a hematopoietic precursor, but are often overlooked in hematopoiesis models because mast cells reside in the tissue.

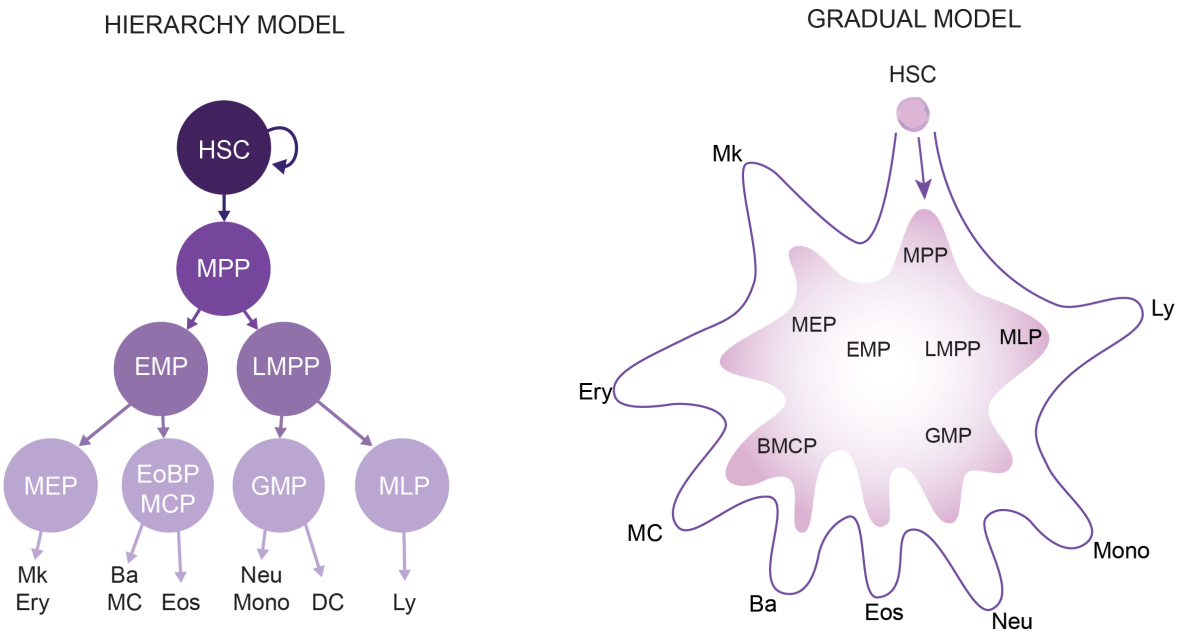

FIGURE 3. Two models describing hematopoiesis, traditionally depicted in a hierarchy model, but recently revised into a gradual model. Adapted from (Grootens et al., 2018).

Mice studies showed for the first time that mast cells arise from bone marrow progenitors (Kanakura et al., 1988; Kitamura et al., 1977) and from early precursors in the embryo (Sonoda et al., 1983; Tsai and Orkin, 1997). Mast cells derived from the embryonic yolk sac are the first skin and connective tissue mast cells in the embryo (Gentek et al., 2018; Li et al., 2018). Bone marrow-derived mast cells gradually replace yolk sac-derived mast cells in adults. Whether mast cells derive directly from the HSC or through intermediate progenitors has long been discussed. The bone marrow in mice contains committed mast cell progenitors (Chen et al., 2005; Jamur et al., 2005), as well as bipotent progenitor basophils-mast cell progenitors (BMCPs), first shown in spleen (Arinobu et al., 2005) and recently in bone marrow (Dahlin et al., 2018).

So far, a unipotent MCP has not been defined in the human bone marrow. However, cultures of human $CD34^+$ bone marrow progenitor give rise to mast cells in vitro (Kirshenbaum et al., 1991) and in vivo (Födinger et al., 1994). As mentioned before, basophils develop from sorted $CD34^+$ $CD203c^+$ cell from the bone marrow when cultured in multilineage stimulating medium, however addition of SCF also gives mast cell colonies as well as eosinophil and macrophage/neutrophil colonies (Buhring et al., 1999). In addition, $CD203c^-$ sorted cells also give mast cell colonies, suggesting that this marker is not specific for mast cell progenitors. Furthermore, $CD34^+$ $CD117^+$ $CD13^+$ progenitors from the bone marrow give mast cell-monocyte colonies (Kirshenbaum et al., 1999).

Circulating $CD34^+$ progenitors in the peripheral blood also give rise to mast cells when cultured in vitro, further specified to be a mast cell progenitor (MCP) that expresses $CD34^+$ $CD117^{int/hi}$ $Fc\varepsilon RI^+$ and comprise about 0.005% of blood mononuclear cells (Dahlin et al., 2016; Maaninka et al., 2013). MCPs lack expression of CD45RA, which places them in the classic CMP gate, but this does not necessarily mean that they are derived from the CMP. The classic CMPs gate is likely not identifying one progenitor state, as others also identified a subpopulation with eosinophil progenitors within the CMP population (Mori et al., 2009). Surface expression of integrin β7 on MCPs regulates migration from the blood to peripheral tissues, where mature mast cells take lifelong residence (Gurish et al., 2001).

Human umbilical cord blood provides another source to study mast cell development. Cord blood contains more multilineage progenitors compared with adult bone marrow (Notta et al., 2016). However, conserved key regulators in both tissues, makes it an interesting source for progenitor studies (Zheng et al., 2018). Single-cell culture experiments of cord blood progenitors suggest that MCPs are enriched in the $CD34^+$ $CD38^+$ $HLA\text{-}DR^-$ cell fraction. However, depending on the added cytokines mast cells colonies also develop from $CD34^+$ $HLA\text{-}DR^+$ and $CD34^+$ $CD38^-$ fractions (Kempuraj et al., 1999).

As mentioned, the transcriptional profile of mature mast cells is unique compared with basophils. Whole transcriptome analysis of blood MCPs shows the expression of several mast cell-associated genes, including tryptase, carboxypeptidase, and serglycin, which are not highly expressed in basophils (Dahlin et al., 2016).

1.4 IN VITRO MAST CELL DIFFERENTIATION

In vitro differentiation of mast cells provides more insight into necessary cytokines and growth factor signaling. Established culture protocols typically start with enriched $CD34^+$ cells from peripheral blood or cord blood and give mature mast cells after 7-8 weeks of culture. It is well established that SCF, the ligand for the KIT receptor, is important for obtaining fully maturate human mast cells (Irani et al., 1992; Kirshenbaum et al., 1992; Mitsui et al., 1993; Valent et al., 1992). The combination of SCF with IL-6, binding to the IL-6 receptor (IL-6R/CD126) gives significant more mast cells after 7 weeks of culturing (Saito et al., 1996). However, others showed a reduction in mast cell numbers when cultured with IL-6 and SCF (Kinoshita et al., 1999). The combination of SCF with IL-3, binding to the IL-3R (CD123), shows beneficial effects on the culture outcome, whereas IL-3 alone does not give mature mast cells with tryptase-expressing granules (Irani et al., 1992; Kirshenbaum et al., 1992). The changes in the surface expression of several markers provide a method to follow in vitro mast cell maturation from $CD34^+$ cells. Cultures of $CD34^+$ progenitors from cord blood in medium with SCF and IL-6 results in reduced expression of CD34, CD123, and CD203c over the course of 7 weeks. In contrast CD63 expression increases and is highly expressed after 7 weeks (Schernthaner et al., 2005). Addition of IL-4 results in upregulation of functional FcεRI and complement receptors CR1 (CD35) and C5aR (CD88) (Schernthaner et al., 2005; Xia et al., 1997). Therefore IL-4 is commonly added during the last days of culture in order to get higher FcεRI expression for studying mast cell activation. However, prolonged exposure to IL-4 downregulates CD117 and inhibits mast cell development (Nilsson et al., 1994b)

Other mast cell culture protocols start with different pre-selected cells, such as $CD133^+$ progenitors from cord blood. After 12 weeks, the cultured mast cells express CD117, CD13, CD33, and FcεRI, but also cytokine receptors CD123, IL-5R, and GM-CSFR, expressed on eosinophils and basophils (Dahl et al., 2004). Following the redefined hematopoiesis models, $CD133^+$ expression is specific for MPPs and the lymphoid-myeloid lineage, whereas erythroid-myeloid lineages that develop into eosinophils and basophils, and likely mast cells, are $CD133^{low}$ cells (Görgens et al., 2013). Thus, mast cells that differentiate in this culture protocol arise from an early progenitor as this protocol discards $CD133^{low}$ progenitors.

1.5 MAST CELL FUNCTION

The most studied function of mature mast cells is their response through IgE-mediated degranulation. Crosslinking by an allergen of two IgE molecules that are interacting with two FcεRI molecules results in the activation of downstream pathways and release of the content of the granules. Mast cells also get activated independently of IgE, through complement receptors, toll-like receptors, or other inflammatory cytokines such as chemokines and interleukins.

Mast cell granules, also called secretory lysosomes, form in the cytoplasm of the cell. They bud of from intracellular compartments such as the lysosomes, and therefore carry different proteins in the membrane compared to the cell surface. As a consequence, when the granule fuses with the cell surface membrane, this increases cell surface expression of certain

tetraspanins such as CD63 (Knol et al., 1991). IgE crosslinking of FcεRI on mast cells also results in upregulation of CD203c (Hauswirth et al., 2008). CD63 and CD203c are commonly used as basophil activation markers (Ocmant et al., 2007).

Mast cell granules contain several pre-stored mediators, including amines (e.g. histamine and serotonin), proteases (e.g. tryptase, chymase), lysosomal enzymes (e.g. β-hexosaminidase), cytokines (e.g. TNF, IL-4) and proteoglycans (e.g. heparin). Mast cells release these mediators in a first and immediate response within seconds to minutes after activation. Subsequently, mast cells release enzymatically synthesized lipid mediators (e.g. leukotriene C4, prostaglandin D2 and platelet activating factor). In a delayed response, mast cells release de novo synthesized proteins including cytokines, chemokines, growth factors and interferons (Moon et al., 2014). Depending on what the mast cells recognize, they release different mediators. Activation through FcεRI results in degranulation and the subsequent release of mediators, whereas activation through for example toll-like receptors leads to the production and release of cytokines and chemokines. After degranulation, mast cells produce new granules and continue to survey in the specific tissue of their residence (Xiang et al., 2001). Mast cells often reside nearby blood vessels where they can increase vascular permeability through mediators such as histamine and VEGF (Abraham and St John, 2010). Mast cells also play a role in smooth muscle cells contraction in organs as the respiratory and gastrointestinal tract and exist in the nervous system (Abraham and St John, 2010). All these processes mediated by the release of mast cell mediators are important for pathogen surveillance, antipathogen reactions and other mechanisms of eliminating microorganisms from the host as an innate immune response. Interestingly, mast cells can also activate the adaptive immune system directly by presenting antigens to T-lymphocytes, and indirectly by activating other antigen presenting cells such as dendritic cells (Carroll-Portillo et al., 2015; Lotfi-Emran et al., 2018).

1.6 THE KIT RECEPTOR

The KIT receptor is part of the family of type III receptor tyrosine kinases that includes macrophage colony-stimulating factor 1 receptor (CSF-1R), the platelet-derived growth factor receptor (PDGFR), and the FMS-like tyrosine kinase 3 (FLT3). These cell-surface receptors have a similar structure, including an extracellular immunoglobin-like domain, a transmembrane domain, the juxtamembrane region and the cytoplasmic kinase domains. Activation of the receptor through ligand binding is a tightly regulated process, triggering dimerization, kinase activation, and phosphorylation of downstream targets (Schlessinger, 2000). As mentioned, the KIT receptor is important for mast cell differentiation. Other mast cell functions regulated by KIT and SCF are migration (Nilsson et al., 1994a), survival (Moller et al., 2005), adhesion (Dastych and Metcalfe, 1994), and potentiation of FcεRI activation (Bischoff and Dahinden, 1992). Non-mast cell mediated processes that require KIT signaling are pigmentation, reproduction, gut motility, functioning nervous system, cardiovascular system, and lung tissue integrity (Lennartsson and Ronnstrand, 2012).

Binding of SCF results in dimerization of the receptor and subsequent activation of the intracellular kinase domains (figure 4). KIT activation results in a conformational change

enabling activation of other signaling molecules that initiate intracellular signal transduction pathways. Downstream signal transduction pathways of KIT create a complex signaling network. Activated pathways include the PI3-kinases/mTOR pathway, JAK-STAT signaling, and MAP Kinase pathways. These pathways function in mast cell survival, proliferation, activation, and migration (Lennartsson and Ronnstrand, 2012). Expression of oncogenic mutations in KIT causes SCF-independent phosphorylation and potentiates several human malignancies, including systemic mastocytosis, which is introduced in the next section. More than 500 different mutations are described in the *KIT* gene, of which most located in exon 11 and 17 (Lennartsson and Ronnstrand, 2012). However, the exact mechanism behind constitutive KIT activation is not fully understood.

1.7 SYSTEMIC MASTOCYTOSIS

SM is a rare disease and classified as myeloid neoplasm by the World Health Organization (WHO). The primary actors in this disease are clusters of aberrant, or neoplastic mast cells. Increase in mast cell mediators are the cause several of the symptoms these patients suffer from. Symptoms include flushing, headache, dizziness, abdominal pain, diarrhea, fatigue or anaphylaxis (Gülen et al., 2016). Symptoms are often not only physical since most of the patients experience a psychosocial influence in their daily live, reflecting on their relationships, work, and family (Jensen et al., 2019).

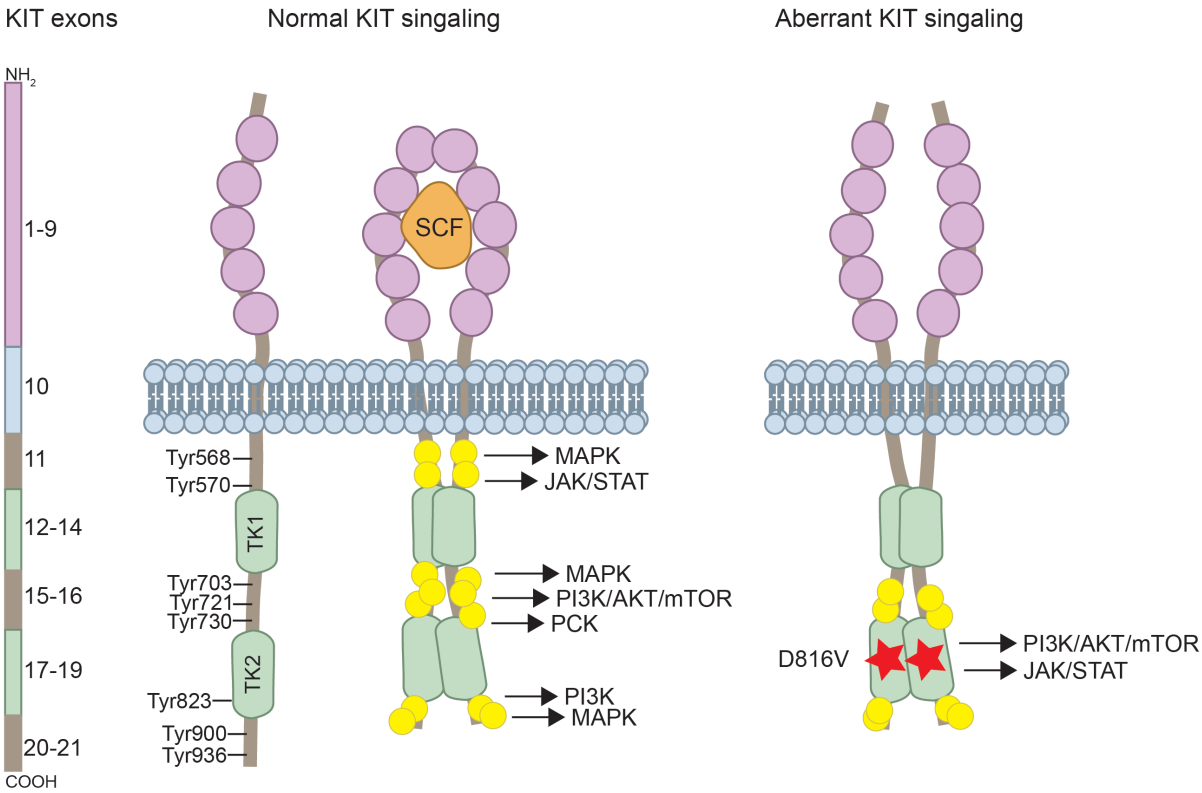

FIGURE 4. Schematic structure of the KIT receptor. Binding of SCF results in dimerization, tyrosine kinase (TK) activation and subsequent phosphorylation of the eight tyrosines (Tyr) that induces downstream signaling pathways. The mutation D816V results in activation without binding of SCF.

The most visible symptoms are typical brown or red lesions in the skin caused by mast cell clustering. Mast cells can also accumulate in other organs, including the bone marrow, spleen, liver, intestine, or lymph nodes. Infiltration of the skin only is named cutaneous mastocytosis (CM) and the accumulation of mast cells in other organs is named systemic mastocytosis (SM). A third rare subcategory identifies localized mast cell tumors or mast cell sarcomas (Valent et al., 2017). The prevalence of mastocytosis is estimated at 0.5-1 per 10,000 adults in central Europe (Cohen et al., 2014; van Doormaal et al., 2013).

1.7.1 DISEASE CLASSIFICATION

The WHO provides guidelines for classification of subtypes within the three main variants of mastocytosis (Arber et al., 2016). CM is more frequently seen in children and resolves often spontaneously around puberty (Hartmann et al., 2016). The type of skin lesions, the distribution, and the disease onset further classifies CM into maculopapular CM (also known as urticaria pigmentosa), diffuse CM, and mastocytoma of the skin (figure 5). Maculopapular lesions are the most common form and are present in about 70% of patients with adulthood-onset of mastocytosis. In adults, lesions usually start to develop around the thigh and can spread to other body parts over the years. Most adult patients have mast cells infiltrates in the bone marrow, which corresponds to the systemic disease. The major criterion for SM describes dense infiltrates of mast cells. Minor criteria describe more specific characteristics including spindle shaped mast cells, a mutation in the KIT receptor, aberrant CD2/CD25 expression on mast cells, and increased serum tryptase (table 1). B-and C-findings further divide SM into indolent SM (ISM), smoldering SM (SSM), SM with an associated hematological neoplasm (SM-AHN), aggressive SM (ASM) and mast cell leukemia (MCL) (table 2).

Immunohistochemistry staining for CD25 and tryptase identifies aberrant mast cells (figure 6). ISM is the most prevalent subtype of SM and symptoms are mainly mast cell mediator-related without organ dysfunction. ISM patients are significantly younger at diagnosis compared with patients with more severe subtypes and usually have more skin lesions and gastrointestinal symptoms. The overall survival of ISM patients is not different compared to the healthy population (Lim et al., 2009). SSM is more severe compared with ISM shown by inferior survival, but more favorable compared with ASM and MCL (Tefferi et al., 2019). SSM is identified by B-findings, whereas C-findings identify ASM and MCL (table 2). In addition, SM patients can present an associated hematologic neoplasm (AHN).

FIGURE 5. Cutaneous forms of mastocytosis are categorized into maculopapular (A), diffuse (B), and mastocytoma (C). Reprinted with permission from Elsevier, original from Hartmann (2016).

TABLE 1. WHO criteria for diagnostics of patients suspected of systemic mastocytosis. The diagnosis is SM when patients have at least 1 major and 1 minor, or 3 minor criteria (Valent et al., 2017).

Criterion	Description
Major	More than one dense infiltrate of mast cells (>15) in at least one tissue that is not skin, by immunohistochemistry
Minor	>25% of the mast cells are spindle-shaped
	Presence of the *KIT* mutation D816V in bone marrow or peripheral blood
	Mast cells express CD2 and/or CD25
	Elevated total serum-tryptase levels of >20 ng/ml

TABLE 2. WHO definition and criteria of B-findings and C-findings in SM. Diagnosis with the presence of 2 B-findings and no C-findings is ISM, more than 2 B-findings and no C-findings is SSM, whereas more than 1 C-finding is ASM or MCL (Valent et al., 2017).

Criterion	Description
B-findings	Indicate a high burden of mast cells and expansion of the neoplastic process into multiple hematopoietic lineages, without visible impairment of organ function 1 Mast cell infiltration grade in the bone marrow >30% by histology and the basal serum tryptase level is >200 ng/mL 2 Hypercellular bone marrow with loss of fat cells, discrete signs of dysmyelopoiesis without substantial cytopenias or WHO criteria for an MDS or MPN 3 Organomegaly: palpable hepatomegaly, palpable splenomegaly, or palpable lymphadenopathy (>2 cm) without impaired organ function
C-findings	Are indicative of organ damage produced by mast cell infiltration 1 Cytopenia(s): absolute neutrophil count <1,000/μL or hemoglobin <10 g/dL or platelet count <100,000/μL 2 Hepatomegaly with ascites and impaired liver function 3 Palpable splenomegaly with associated hypersplenism 4 Malabsorption with hypoalbuminemia and weight loss 5 Skeletal lesions: large-sized osteolyses with pathologic fractures 6 Life-threatening organ damage in other organ systems that is caused by local mast cell infiltration in tissues

AHNs are most frequently other myeloid neoplasm such as myelodysplastic syndromes (MDS), chronic myelomonocytic leukemia (CMML), forms of, or acute myeloid leukemia (AML), or myeloproliferative neoplasms (MPNs), but can also include lymphoid hematological malignancies (Valent et al., 2017). A reduced median survival of 2-3 years after diagnosis marks more advanced subtypes, whereas median survival in MCL is only 2 months after diagnosis (Lim et al., 2009).

The third mastocytosis category describes the presence of mast cell sarcomas, manifesting as an isolated destructive mass. These tumors are extremely rare with about ten cases reported in the literature. Their limited resemblance to normal mast cells makes identification and correct diagnosis rather difficult. In addition, patients with mast cell sarcoma do not fulfill the major criteria and usually do not carry the same mutation in *KIT* as most SM patients (Ryan et al., 2013).

1.7.2 ABERRANT MAST CELL MEDIATORS

As mentioned, patients with ISM experience symptoms that are the result of mast cell mediator release, with the most severe reaction being an anaphylactic reaction. A study among 84 Stockholm patients including different SM subtypes showed that 36 of 84 (43%) patients experience at least one anaphylactic episode, mainly caused by wasp stings (Gulen et al., 2014). Several mediators show a degree of elevation in SM patients, of which a serum tryptase level >20 ng/mL is the only one that indicates a minor diagnostic criterion. The serum tryptase levels correlates with the percentage of mast cell infiltrates in the bone marrow (Schwartz et al., 1995; Sperr et al., 2002). In addition, high serum tryptase is associated with poor prognosis and subsequent disease progression (Matito et al., 2013). Other diseases can also present increased tryptase levels, such as MDS, MPN, AML, CML or renal failure (Sirvent et al., 2010; Valent et al., 2014). Another prominent mast cell mediator found in the majority of SM patients is serum IL-6 (Brockow et al., 2002; Theoharides et al., 2002). IL-6 presents an early marker for disease progression in ISM (Mayado et al., 2016). Furthermore, a recent study showed that aberrant signaling of oncogenic KIT induces IL-6 production (Tobío et al., 2019).

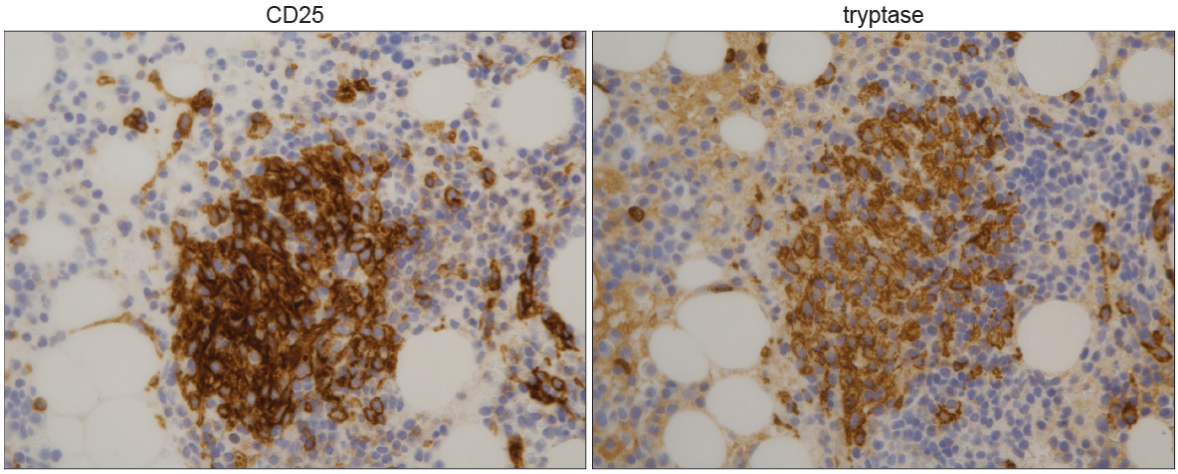

FIGURE 6. Immunohistochemistry staining of bone marrow biopsies showing mast cell infiltrates using CD25, and tryptase in the same biopsy. Photo credits Igor Schliemann, Karolinska University Hospital.

Other known mast cell mediators in SM are histamine and several lipid mediators, such as prostaglandins and leukotrienes and platelet activating factor (Butterfield et al., 2018).

1.7.3 EXPRESSION PROFILE OF ABERRANT MAST CELLS

Mast cells in SM often present aberrant protein expression, detectable by flow cytometry, also used in the clinic as complementary method to immunohistochemistry. For example, CD117 is informative as immunohistochemistry marker to detect mast cell clusters in tissue biopsies, providing essential information on disease progression. Flow cytometry analysis of CD117 in combination with FcεRI provides the percentage of mast cells in a bone marrow aspirate. Comparing surface expression of these markers in SM subtypes showed comparable expression of CD117 and FcεRI in ISM, SM-AHN, and controls (Teodosio et al., 2010). However, lower expression of CD117 and FcεRI is often observed in MCL and ASM.

In the last two decades, multiple aberrant markers are identified on SM mast cells in the bone marrow using flow cytometry. These markers are absent or expressed at low levels on normal bone marrow mast cells (figure 7). Currently only CD2 and CD25 contribute to diagnosis (Escribano et al., 2001; Jabbar et al., 2014; Teodosio et al., 2015).

Diagnostic markers. CD2 and CD25 (IL-2Rα) are the first specific markers described for SM in a cohort of 10 ISM patients (Escribano et al., 1998b). Whereas CD25 is commonly present on aberrant mast cells in SM patients, aberrant CD2 expression is not always present, particularly in AdvSM, making it a less reliable maker compared with CD25 (Morgado et al., 2012). Both antigens are commonly known as lymphoid markers, CD2 as early marker for T cells, whereas CD25 is a marker for regulatory T cells and activated T cells. Interestingly, CD2 and CD25 expression is not specific for mast cells in SM since mast cells in a subset of patients without any history of SM also express CD2 and CD25. These patient however, have other neoplasms including lymphoblastic leukemia and plasma cell neoplasm (Cherian et al., 2016).

Advanced SM markers. Another lymphoid marker expressed by neoplastic cells in some lymphoid neoplasms is CD30 (Ki-1 antigen) (Sotlar et al., 2011). In SM, CD30 expression is specific for mast cells in AdvSM patients, providing a biomarker of aggressive disease variants. Similarly, CD52 expression marks neoplastic mast cells of AdvSM patients, but is absent on normal and ISM mast cells (Hoermann et al., 2014a).

Activation markers. Expression of several activation markers on neoplastic mast cells may reflect the increase in activation of the mast cells. SM patients have increased CD63 surface expression on mast cells (Escribano et al., 1998a). Increase in CD203c expression on neoplastic mast cells is highest in ISM (Hauswirth et al., 2008). Additionally, SM patients have higher expression of CD69, but this varies between patients (Diaz-Agustin et al., 1999; Jabbar et al., 2014). CD69 is a T-cell activation maker that is also expressed on basophils and eosinophils upon degranulation, similar to CD63 but with a slower response (Yoshimura et al., 2002). Another T cell and eosinophil activation marker, CD44, is increased on mast cells of all SM subtypes (Matsumoto et al., 1998; Sano et al., 1998). In addition, the level of soluble CD44 is higher in the serum of AdvSM patients compared with healthy controls and CM patients (Mueller et al., 2018).

Neoplastic markers. Other markers are reported on neoplastic progenitor cells in myeloid neoplasms, including CD33 and CD123. Normal mast cells and basophils express low levels of the myeloid marker CD33 (Siglec-3), whereas expression is higher on SM mast cells (Krauth et al., 2007). CD33 expression is found in early differentiation and is an aberrant marker on $CD34^{+}$ $CD38^{-}$ neoplastic progenitor cells and basophils in myeloid neoplasms (Florian et al., 2006). Normal stem and progenitor cells, as well as basophils and dendritic cells express the IL-3R, CD123. CD123 is detectable on neoplastic mast cells of all ASM patients, whereas only a subset of the ISM and SM-AHN patients have CD123 expression on their mast cells. However CD123 is not detected in MCL (Pardanani et al., 2016b).

Other markers. The pan leukocyte marker CD45, also expressed on normal mast cells has increased expression on SM mast cells (Chisholm et al., 2015). The majority of SM patients have bright expression of the complement receptor type I, CD35, on their mast cells, whereas normal mast cells lack CD35 expression. (Escribano et al., 1998b; Jabbar et al., 2014). Other complement receptors CD88, CD59 and CD11c also have higher expression on SM mast cells (Nunez-Lopez et al., 2003). Interestingly, tumor cell marker PD-L1 is also present in neoplastic mast cells in the bone marrow and skin (Rabenhorst et al., 2016)

Gene expression. The gene expression of neoplastic mast cells from the bone marrow is studied using microarrays (D'Ambrosio et al., 2003; Teodosio et al., 2013). As expected, high tryptase expression in mononuclear cells distinguishes SM patients from healthy controls (D'Ambrosio et al., 2003). Isolated bone marrow mast cells from ISM and ASM patients showed a common expression profile with several deregulated transcripts related to the immune response, as well as KIT and tryptase (Teodosio et al., 2013). However, mast cells from ISM patients have some specific upregulated proteins that are involved in signaling pathways and degranulation.

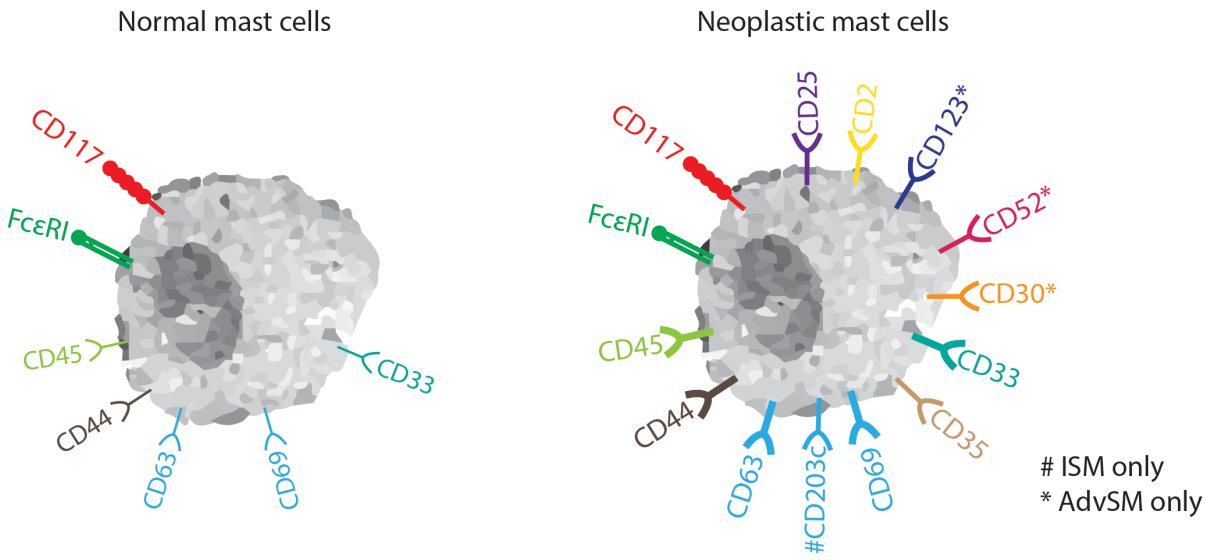

FIGURE 7. Neoplastic mast cells in SM express several aberrant surface makers compared to normal mast cells. CD45, CD44, CD63, CD69, and CD33 are expressed on normal mast cells, but have increased expression on neoplastic mast cells.

ASM mast cells have more altered genes involved in apoptosis, cell cycle, proliferation, and DNA repair. It is not reported whether these transcripts are associated with additional mutations found in ASM.

It is not clear why these markers are elevated and what they have in common. Some of the aberrant markers are undetectable on normal mast cells, whereas other aberrant markers are present at low levels in normal mast cells and show an increase in neoplastic mast cells. Interestingly, signaling of oncogenic KIT-activated RAS and STAT induces expression of CD52 and CD44 (Hoermann et al., 2014a; Mueller et al., 2018). The complex signaling network of KIT likely causes aberrant expression when KIT is mutated, but there is only limited evidence for this.

1.7.4 *KIT* MUTATIONS

The most common mutation in SM, included as minor criterion for SM diagnosis, is the mutation in exon 17 of the *KIT* gene affecting the aspartic acid (D) at amino acid position 816. In SM this mutation results in the change to valine (D816V), but there are other, less common changes at this position including D816F, D816H, D816I, and D816Y (Arock et al., 2015). The D816V mutation leads to SCF-independent activation of the intracellular tyrosine kinase domain of KIT, which induces phosphorylation of downstream pathways. (Furitsu et al., 1993). The STAT5-PI3K signaling pathway is strongly affected by *KIT* D816V (Harir et al., 2008; Xiang et al., 2007). In vitro inhibition of STAT5 abrogates the growth of *KIT* D816V expressing mast cells from SM patients through direct activation of STAT5 by *KIT* D816V (Baumgartner et al., 2009). Similarly, KIT D816V activates mTOR in the PI3K/mTOR signaling pathway. Targeting mTOR with rapamycin inhibits proliferation of *KIT* D816V mast cell but not mast cells with wildtype *KIT* (Gabillot-Carre et al., 2006). In contrast, downstream proteins from the MAPK pathway are not constitutively active in *KIT* D816V mutated cells, showing that not all downstream pathways are affected by this mutation (Chian et al., 2001) (figure 4).

The *KIT* D816V mutation is the most common mutation in adult patients. The majority of these SM patients also carry the *KIT* D816V mutation in skin lesions (Kristensen et al., 2013). In contrast, skin lesions are present in >90% of pediatric patients but only 35% of these patients carry the *KIT* D816V mutation in the skin or bone marrow (Hartmann et al., 2016; Méni et al., 2015). The most common *KIT* mutations in pediatric patients exist in exon 8, 9, and 11, located in the extracellular and juxtamembrane domain of KIT (Bodemer et al., 2009). These mutations are not exclusively present in pediatric mastocytosis, but are also reported in patients with gastrointestinal stromal tumors (GIST). *KIT* D816V is a somatic mutation and not inherited, whereas other *KIT* mutations are found to be germline mutations, resulting in familial cases of SM and GIST (Hartmann et al., 2005).

A commonly used method to detect the *KIT* D816V mutation is an allele-specific qPCR (Kristensen et al., 2011). Calculating the ratio between *KIT* D816V and wildtype *KIT* alleles gives the percentage mutated *KIT* alleles which provides an estimation of the disease burden. The median *KIT* D816V allele burden in all SM patient is 0.315%, ranging from 0.005-50.183%. The *KIT* D816V allele burden correlates with SM subtype, with significant reduced probability

of survival with an allele burden of ≥2% (Hoermann et al., 2014b). Follow-up assessment of the ISM patients shows a stable mutation burden in patients with stable clinical course, whereas an increase in mutation burden is present in patients with disease progression. The *KIT* D816V mutation is not detected in about 20-30% of patients with MCL, whereas alternative mutations at position D816 are reported (Jawhar et al., 2017).

The peripheral blood is another source for detection of the *KIT* D816V mutation. Since mast cells usually do not circulate in the blood, the detection of the mutation comes from other hematopoietic cells, mature cells or progenitors. Indeed, several studies reported the *KIT* D816V mutation in mature blood cells of SM patients other than mast cells, showing multilineage involvement. *KIT* D816V is present in lymphocytes, monocytes, neutrophils, basophils, and eosinophils (Garcia-Montero et al., 2006; Kocabas et al., 2005; Mayado et al., 2018; Pardanani et al., 2003; Yavuz et al., 2002). Furthermore colonies from erythroid progenitors and granulocyte/monocyte precursors from AdvSM patients carry *KIT* D816V (Afonja et al., 1998; Jawhar et al., 2015). A report on associated neoplasms detected the mutation in neoplastic $CD15^+$ myeloid cells (Sotlar et al., 2008). As expected, $CD34^+$ sorted hematopoietic progenitors carry *KIT* D816V in patients with different SM subtypes (Garcia-Montero et al., 2006; Mayado et al., 2018). However, it remains to be confirmed if *KIT* D816V is present in specific hematopoietic stem and progenitor cells.

Interestingly, it seems that the *KIT* D816V mutation is not restricted to the hematopoietic system, since mesenchymal stromal cells can carry the mutation as well (Garcia-Montero et al., 2016; Nemeth et al., 2015). In addition, CCL2, a proangiogenic cytokine released by aberrant mast cells, promote alterations of the tumor microenvironment, tested in vivo and in vitro (Greiner et al., 2017). Together these findings suggest that aberrant KIT signaling in mast cells as well as the stroma cells affects the bone marrow niche.

Not much is known about the effect of *KIT* D816V on other cells than the mast cell. Current knowledge is based on primary mast cells from patient samples and human mast cell lines that harbor the *KIT* D816V mutation; HMC-1.2, derived from a patient with MCL (Sundstrom et al., 2003), and ROSA *KIT* D816V cells, cord blood derived mast cells transduced with a *KIT* D816V construct (Saleh et al., 2014). ROSA *KIT* D816V cells injected in a mouse model results in engraftment of the cells in the bone marrow, spleen, liver and peripheral blood (Bibi et al., 2016). This model provides an interesting tool to study the effect of potential drugs, but will not provide more insight into disease etiology or pathogenesis. Other transgenic animal models in mice and zebra fish enables studies on *KIT* D816V in development (Balci et al., 2014; Gerbaulet et al., 2011; Weidemann et al., 2017; Zappulla et al., 2005). In mice, inducing the murine homologue of *KIT* D816V, *Kit* D814V, in germline cells results in perinatal lethality due to hyperproliferation of the erythroid lineage. Introducing the mutation after birth gives mast cell infiltrates in the skin, lymph nodes, spleen, forestomach, intestinal mucosa and liver (Gerbaulet et al., 2011). These findings might not be directly translatable to humans, but it might explain why the *KIT* D816V is a somatic mutation and not inherited.

Whereas mastocytosis is not reported to occur naturally in mice, mast cell tumors present the most common skin tumors in dogs. Similar to human mast cell sarcoma, sequencing data of

the kinase domain of canine *KIT* does not present any mutations in this domain, whereas activating mutations are found in other exons of *KIT* (Letard et al., 2008; Webster et al., 2006).

1.7.5 ASSOCIATED MUTATIONS

In addition to *KIT* D816V, patients with SM can carry associated mutations, most commonly present in SM-AHN followed by ASM and only rarely in ISM (Pardanani et al., 2016a; Schwaab et al., 2013). The presence of additional mutations is associated with a significantly reduction in overall survival (Schwaab et al., 2013). Most of the associated mutations are known from other myeloid neoplasms (Mian et al., 2013). However, patients with myeloid neoplasms can also carry the *KIT* D816V mutation, as shown in patients with CMML or AML, presenting an associated SM in CMML, but not in AML (Jawhar et al., 2015; Kristensen et al., 2012). The most common associated mutations in SM are *TET2, SRSF2, ASXL1, RUNX1, N/KRAS* and *JAK2*, involved in epigenetic regulation, RNA splicing or cell signaling pathways (Hanssens et al., 2014; Jawhar et al., 2016). The combination of mutations in *SRSF2*, *ASXL1*, *RUNX1* and *EZH2* adversely correlates with overall survival (Jawhar et al., 2016). The combination of *TET2* mutations with *KIT* D816V presents a more aggressive phenotype compared with *KIT* D816V only (De Vita et al., 2014; Soucie et al., 2012). *TET2* has a regulatory role in mast cell differentiation and function (Montagner et al., 2016). Interestingly, analysis of colonies grown in myeloid restricted medium from sorted $CD34^+$ progenitors suggest that the associated mutations *TET2*, *SFSF2* and *ASXL1* are acquired before the *KIT* D816V mutation (Jawhar et al., 2015). It is unknown if *KIT* D816V and associated mutations arise from a single clone or whether they arise separately. In addition, the presence of a neoplastic stem cell is shown in other myeloid neoplasms, making them stem cell diseases. A similar neoplastic stem cell is described in MCL patients, but is thus far not reported in other subtypes of SM patients (Eisenwort et al., 2019; Valent et al., 2017).

1.8 MAST CELLS AS TARGETS OF TREATMENT

There is currently no curative treatment option for SM. Patients are mainly receiving medication that targets mast cell mediators, such as anti-histamines, leukotriene inhibitors, anti-interferon and steroids. In the recent years, new treatment concepts have been developed and tested. However, since SM is heterogeneous, there is not one best treatment option and therefore the field is moving more towards personalized medicine. Some of the recently developed treatments that show promising results are discussed below.

Tyrosine kinase inhibitors (TKI). Since the main mutation in SM is located in the KIT receptor, it makes sense to target the receptor directly, thereby blocking all downstream signaling. The TKI imatinib, initially designed for treatment of CML to target the BCR-ABL fusion protein, targets the KIT receptor (Druker et al., 2001). Mechanistic studies showed that imatinib inhibits mast cell lines with the V560G mutation, but not with the D816V mutation and is therefore not used for SM patients (Ma et al., 2002). The conformational change in the kinase domain of KIT caused by the D816V mutation makes the binding site for imatinib inaccessible (Laine et al., 2011). However, imatinib gives a good response in SM patient without mutations

in exon 17 of the KIT receptor (Alvarez-Twose et al., 2017). New TKI's are under development since. Multi-TKI, midostaurin (PKC412), inhibits wildtype KIT as well as *KIT* D816V (Gleixner et al., 2006). Midostaurin is evaluated in clinical trials and is recently approved by the FDA and EMA for treatment of ASM (Gotlib et al., 2016; Kasamon et al., 2018). Another promising TKI is currently tested for safety in a phase II clinical trial for treatment of ISM and ASM is avapritinib (BLU-285; NCT03731260 and NCT03731260) (Lubke et al., 2019).

Downstream signaling pathways. Small molecular inhibitors can target activated signaling downstream of KIT. The JAK/STAT signaling pathway provides an interesting target since *KIT* D816V directly activates it (Baumgartner et al., 2009). JAK inhibitor ruxolitinib is tested in SM patients and improved the quality of life, though does not affect mast cells infiltration or tryptase levels (Dowse et al., 2017; Yacoub and Prochaska, 2016). However, multi-kinase inhibitor R763 shows promising effects by growth inhibition of neoplastic mast cells through inhibiting STAT5 phosphorylation (Peter et al., 2018). Targeting the PI3K/mTOR is less effective. Targeting mTOR is promising in human cell-lines, however the oral mTOR inhibitor everolimus does not give clinical improvement in patients (Gabillot-Carre et al., 2006; Parikh et al., 2010).

Surface proteins. As mentioned previously, neoplastic mast cells in SM express several aberrant proteins, making them possible targets. So far only a few surface proteins are used in the development of targeted therapies. The monoclonal antibody brentuximab vedotin targeting CD30 is tested in a few patients, showing a reduction of disease burden in 2/4 patients (Borate et al., 2016). The low toxicity of this drug is promising and the efficacy is further investigated in a phase II clinical trial (NCT01807598). The antibody alemtuzumab targeting CD52 induces favorable effects in cell lines and mice, but is not evaluated in clinical studies so far (Hoermann et al., 2014a). Gemtuzumab ozogamicin targets CD33 with a monoclonal antibody conjugated to a cytostatic agent, commonly used for $CD33^{+}$ AML and successful in treatment of a MCL patient with no response to other therapies (Krauth et al., 2007). Aberrant CD123 expression on neoplastic mast cells provides another promising target for monoclonal antibodies, of which SL-401 is currently tested in clinical trials for efficacy and safety for treatment of advanced myeloproliferative neoplasms including SM (NCT02268253) (Frankel et al., 2014; Pardanani et al., 2016b)

Epigenetics. Research into epigenetics is increasing in the research areas of different kind of malignancies. Histone deacetylase inhibitor vorinostat (SAHA) selectively induces apoptosis in *KIT* D816V mutated cells in vitro as well as ex vivo using primary neoplastic mast cells (Lyberg et al., 2017)

Allogeneic hematopoietic cell transplantation. Currently the only method available to remove mutated progenitor cells for advanced hematological diseases is through the complete replacement of all hematopoietic stem progenitor cells by allogeneic hematopoietic cell transplantation from a genetically similar donor. From 1990 several patients with AdvSM receiving an allogeneic transplant published in several case studies (Ustun et al., 2014). Clinical outcome depends on the SM subtype but 51% of the subjects are progression-free after 3 years. Responding patients have significant lower percentages of mast cells in the bone

marrow as well as lower serum tryptase levels. Thus, allogeneic transplantation provides a possible treatment option for patients with advanced SM. However, it has to be noted that most of the SM patients are not eligible for stem cell transplantation due to age and or comorbidities.

2 AIMS

The overall aim of this thesis is to gain more insights into mast cell development in health and to compare it to systemic mastocytosis thereby increasing basic understanding and identify novel targets for potential therapies in systemic mastocytosis.

The specific aims were:

Study I To investigate the presence of mast cells in the blood of systemic mastocytosis patients

Study II To study the role of SCF and KIT-signaling in mast cell development

Study III To identify the origin of the *KIT* D816V mutation within hematopoietic stem and progenitor cells of systemic mastocytosis

Study IV To identify a mast cell-basophil bone marrow progenitor

3 METHODOLOGICAL APPROACHES

This chapter briefly describes the main methods and materials used in this thesis. More details are provided in the materials and methods section of each study.

3.1 ETHICAL CONSIDERATIONS

The Ethical committees of Stockholm and Uppsala provided permission for the use of human bone marrow and peripheral blood samples. Sample collection was in accordance with the World Medical Association Declaration of Helsinki. Before donation, patients provided oral and written consent to qualified clinicians involved in the studies.

3.2 HUMAN SAMPLES

We collected peripheral blood samples from patients diagnosed with systemic mastocytosis in study **I** and **II,** and from patients with chronic myeloid leukemia (CML) or gastrointestinal stromal tumors (GIST), of which some were treated with imatinib in study **II**. Patient samples were compared with anonymous healthy controls from buffy coats or donations at the blood bank.

For study **III** and **IV** we collected bone marrow samples from patients under evaluation for mastocytosis. Control samples in study **III** included bone marrow from healthy volunteers, and patients that were suspected of mastocytosis but did not fulfill the criteria for the diagnosis. Three of the control subjects had urticaria pigmentosa and where therefore classified with CM. Study **III** included 27 patients and 9 control subjects. Study **IV** includes 10 bone marrow samples, of which five from patients diagnosed with different mastocytosis subtypes and five from healthy volunteers.

3.3 SAMPLE PROCESSING

Cells from bone marrow and peripheral blood were isolated using a standardized method for cell isolation based on lysis of erythrocytes by ammonium chloride potassium (ACK) buffer (study **I**), or PharmLyse (BD Biosciences) (study **I-IV**). In study **II**, mononuclear cells were isolated from buffy coats using Ficoll-Paque PLUS (GE Healthcare). For erythrocyte lysis using ACK buffer, we added 30 mL of ACK buffer to 3 mL of whole blood and incubated for 5 min., followed by several washing steps using FACS buffer (PBS with 2% heat-inactivated Fetal Calf Serum). For lysis with PharmLyse, we added 1.5 volumes of 1x PharmLyse to the sample and incubated for 5 min, after which several washing steps with PBS and FACS buffer further cleaned the sample.

3.4 FLOW CYTOMETRY

We used several multi-color flow cytometry panels for sample analysis (study **I-IV**). Panels were designed based on published studies in which they confirmed the gating strategy of the cell by culture experiments. A combination of lineage markers excluded lymphocytes and/or monocytes: CD14, CD3, CD19, CD4, and/or CD8. Since SM mast cells can express several

aberrant markers (Teodosio et al., 2015), when gating mast cells, only CD14, or a combination of CD14, CD3, and CD19 was used to exclude lineage cells in study **I-III,** and **IV** respectively.

Fluorescent minus one (FMO) controls were used to confirm the antibody staining and to set the gates when analyzing or sorting the samples. The preferred method for gating in all studies was the use an internal/biological control. For example, in study **III** $CD38^+$ cells were gated using $CD38^-$ $CD90^+$ HSCs from the same sample.

3.5 FLUORESCENT-ACTIVATED CELL SORTING

Cells were sorted for different downstream assays using the FACS ARIA I or ARIA Fusion (BD Biosciences). When sorting rare population from bone marrow or blood, we used a two-step sorting protocol. The first sorting round we used the 'yield' precision mask, followed by a 'purity' precision mask at the second round. Sorted cells were checked for purity by recording 20-100 cells that were sorted in sterile filtered FACS buffer after thorough cleaning of the machine. Purity was usually >95%.

For single cell sorting (study **III**) we followed a one-step sorting protocol using the single-cell precision mask at a low flow rate combined with the index-sorting option. The index-sorting option saves the flow cytometry standard (fcs) data for each sorted single cell linked to the specific well. Between 81-86 single cells were sorted into a 96-well plate, leaving several wells as control wells in every plate. Controls included single mast cells from the same sample, at least 3 empty wells, and at least 2 wells with a population of 5-10 cells. Since only one cell was sorted per well directly into lysis buffer, purity cannot be assessed, however, the single-cell sort precision sets very specific restrictions on when to sort a cell.

3.6 CELL CULTURES

In vitro cell cultures in study **II, III,** and **IV** measured cell potential of sorted progenitors. For mast cell potential, cultures were supplemented with a combination of IL-3 (10 ng/mL), IL-6 (10 ng/mL), and SCF (100 ng/mL). However, the high concentration of SCF resulted in internalization of CD117, which makes flow cytometry analysis of the receptor uncertain. In order to allow for flow cytometry analysis, we reduced the concentration of SCF to 5 ng/mL in study **IV**. Addition of EPO (1 IU/mL), and GM-CSF (10 ng/mL) allowed for erythroid and monocyte-granulocyte differentiation of sorted progenitors.

In study **III**, we used colony-forming assays in semi-solid medium to confirm the gating strategy of previously described progenitors, the CMPs, GMPs, and MEPs.

3.7 CELL CULTURE ANALYSIS

We analysed the potential of cultured cells by flow cytometry (study **II-IV**) and histology staining using May-Grünwald Giemsa (MGG) (study **I-IV**). Enzymatic staining using Z-Gly-Pro-Arg-4-methoxy-β-naphthylamide and Fast Garnet GBC visualized tryptase-expressing cells (Harvima et al., 1988). For photos at magnification 100x, the slides were mounted with different mounting solutions. MGG stained slides with Pertex (Histolab) and tryptase-stained slides with SlowFade Gold Antifade mountant (Thermofisher)

Flow cytometry antibody panels identified mature immune cells including mast cells, basophils, monocytes and neutrophils, and erythrocytes. Staining with DAPI or 7-AAD enabled exclusion of dead cells in the cultures.

3.8 *KIT* D816V MUTATION ASSAY

Study **I** and **III** utilize a mutation assay that was adapted from the standardized method in the clinic to analyze patient bone marrow and blood for the *KIT* D816V mutation (Kristensen et al., 2011). In study **I**, we isolated extracted DNA, performed a pre-amplification and performed the qPCR on the cleaned PCR product using the original primers and protocol described by Kristensen et al (2011).

The method was further optimized for single cell analysis in study **III.** The method was optimized in several steps:

- Primer design: the primers that are specific for the *KIT* D816V mutation described by Kristensen et al. (2011) are designed with a mismatch at the 3'-minus 2 position. We found that using a *KIT* D816V primer without mismatch was more specific when using low cell numbers (figure 8).

- Multiplexing: every sorted cell needed a control assay to validate the mutation assay. Using a multiplex qPCR assay allowed us to remove a preamplification step and made the assay less labor intense. We designed a control assay in the stable gene GAPDH, which is not likely to get mutated in disease situations. The designed primers amplified a region flanking exon 8 and intron 8, which allowed to detect DNA from 1 cell and distinguish 1 cell from multiple cells.

- Testing in cells: we used two mast cell-lines for testing, ROSA cells that have wildtype *KIT*, and HMC-1.2 that carry the *KIT* D816V mutation. We used purified DNA for quality control of the primers and sorted 1, 5, 10, and 100 cells for testing the combined sorting and mutation analysis by qPCR.

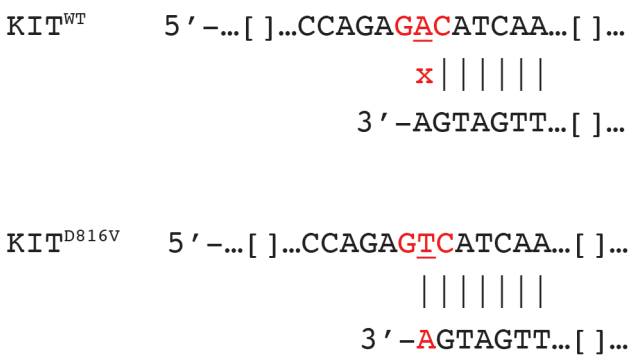

FIGURE 8. Binding of the reverse primer of the mutation assay to wildtype and mutated KIT at position D816

- Cell lysis: to reduce the pipetting steps and risk of contamination, we aimed to sort cells directly into lysis buffer. The lysis buffer contained Qiagen's elution buffer (EB) supplemented with 10mM Tris-Cl (pH 8.5) with 20 mg/mL Proteinase-K (Thermo Fisher Scientific) for 45 min. at 55 °C followed by heat-inactivation of the proteinase for 10 min. at 95 °C.

- Single cell index sorting: we labeled the ROSA and HMC-1.2 cells with different antibodies for CD117, mixed them in a 1:1 ratio and sorted single cells into 96-well plates gated on singlets. After sorting, cells were lysed and subjected to the multiplex assay. We saved the data for each well using the index-sorting option in the software, allowing for a blind testing of the assay (figure 9).

SM patient samples were used for single cell sorting when there was a clear indication that the patient carried the *KIT* D816V mutation. For each patient we aimed to sort between 10-20 96-well plates to get about 1000 cells to for *KIT* D816V qPCR analysis.

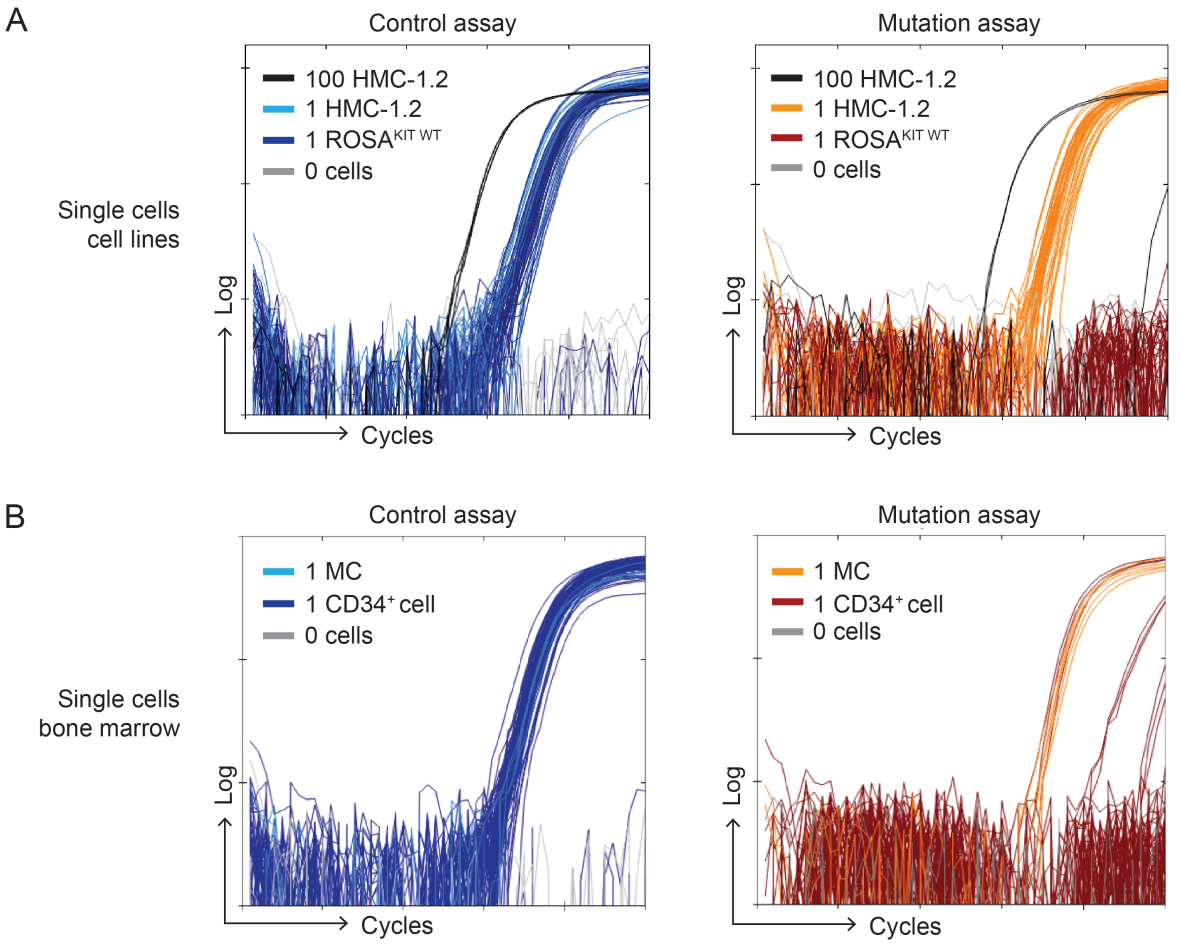

FIGURE 9. Amplification of the control and *KIT* D816V mutation assay in single cells and cell populations from cell-lines (A) and single bone marrow cells (B).

4 RESULTS AND DISCUSSION

This chapter gives an overview of the main findings of each study and discusses their relevance. The figures and tables referred to in the text are found in the corresponding study attached to this thesis.

4.1 CIRCULATING MAST CELLS IN ADVANCED MASTOCYTOSIS (STUDY I)

MCPs, $CD34^{+}$ $CD117^{hi}$ $Fc\varepsilon RI^{+}$ cells, circulate through the blood and mature, $CD34^{-}$ $CD117^{hi}$ $Fc\varepsilon RI^{hi}$ cells, in the tissue. In SM, mast cells can infiltrate and accumulate in different organs, primarily detected in the bone marrow. One subtype of SM is MCL, in which mature mast cells also circulate in the peripheral blood. However, it is unknown if other subtypes of SM can have circulating mast cells. With this in mind we analyzed peripheral blood samples for the presence of mast cell infiltrates.

Using a multi-color flow cytometry panel, we detected circulating $CD34^{-}$ $CD117^{hi}$ $Fc\varepsilon RI^{hi}$ mast cells in four patients that were diagnosed with SM-AHN or ASM (**Figure 1A and 1B in study I**). We did not detect a population of circulating mast cells in ISM or healthy control subjects. We detected the *KIT* D816V mutation in bulk-sorted mast cells carried. Clinical analysis showed that not all circulating mast cells in the four patients had atypical morphology or CD2 expression, and serum tryptase levels did not predict the presence of circulating mast cells (**Table 1 in study I**). Thus, clinical findings other than SM subtype, did not correlate with the presence of circulating mast cells, however, the size of this patient cohort is too small to draw any conclusion about this. One possible explanation for the presence of circulating mast cells in AdvSM patients is that mast cells leak from highly infiltrated tissues since those patients usually have a higher BMMC infiltration rate compared to ISM patients. It is also possible that circulating MCPs that carry the *KIT* D816V mutation mature in the blood and do not migrate into tissues.

Recently, after we published this study, circulating mast cells were reported in a few ISM patients and control subject (<0.001% of the peripheral blood), which was correlated to BMMC load and serum tryptase (Mayado et al., 2018). In our study we did not detect a distinct population of circulating mast cells in ISM patients. We detected a few $CD34^{-}$ $CD117^{hi}$ $Fc\varepsilon RI^{+}$ events (of the total 10^{7} events recorded) in ISM patients and control subjects, however this was by our definition not enough to present a mast cell population.

As mentioned, circulating mast cells are found in MCL, in which the atypical form has < 10% circulating mast cells from total white blood cells. So how do we distinguish between MCL and ASM and SM-AHN in these cases based on peripheral blood? In this study, the percentage of mast cells in bone marrow smears was ≤ 0.5% for all patients, which in MCL is > 20% (**Table 1 in study I**).

Taken together, in this study we demonstrated circulating mast cells in patients with advanced SM subtypes. This finding will not be sufficient to diagnose patients and requires a study in a larger cohort of patients. However detecting mast cells in the blood using flow cytometry presents a useful method to monitor disease progression.

4.2 THE ROLE OF KIT IN MAST CELL PROGENITOR DIFFERENTIATION (STUDY II)

In the second study of this thesis, we continued to study peripheral blood, but with the focus on mast cell progenitors. It is commonly recognized that SCF and KIT signaling are required for the maturation of mast cells and is included into almost all in vitro differentiation protocols. In this study we aimed to assess the role of SCF and KIT signaling in the differentiation of mast cell progenitors. We analysed peripheral blood from patients with SM, CML or GIST, and studied MCP differentiation by culturing $CD34^+$ selected cells or sorted MCPs from of healthy controls.

Similar to our findings in study I, we detected circulating mast cells in a subset of SM patients, but interestingly we also detected circulating mast cells in newly diagnosed and untreated CML patients. These cells were not present in patients that were under treated with imatinib, a drug that disrupts KIT signaling. In contrast, we found that the percentage of blood MCPs is unaffected comparing several patients groups despite imatinib treatment (**Figure 1 in study II**), indicating that a functional KIT might not be necessary for MCP development.

Culture experiments of $CD34^+$ selected cells from blood with addition of IL-3 and IL-6, but without addition of SCF gave rise to pre-mast cells, demonstrating that MCPs can survive without KIT signaling (**Figure 2 in study II**). We showed that IL-3 alone was sufficient for MCPs survival, and found that MCPs expressed CD123 (**Figure 6 in study II**).

Disruption of the KIT signaling by addition of imatinib or anti-SCF to the cultures did not affect MCP survival (**Figure 2 and 3 in study II**). Cultures with IL-3 and IL-6 also showed some maturation of MCPs, measured by decreased CD34 and Integrin β7 expression, and the presence of tryptase expressing granules (**Figure 4 in study II**). However, when IL-3 was replaced after 5 days by SCF for an additional 5-7 days, the cells rapidly matured. Thus, SCF is dispensable for MCP survival and initial maturation, but is required for definitive maturation of mast cells.

Imatinib treatment efficiently depleted the CD117 expressing cells in the CML patients. It is known that imatinib is not effective for treatment of SM patients with the *KIT* D816V mutation because imatinib cannot target this mutation. This shows that mast cells in CML do not carry *KIT* D816V. It is clear on the flow cytometry plot that the population of circulating mast cells had varying CD117 expression without clear separation from $FcεRI^+$ $CD117^-$ cells. CML is known for the presence of basophilia, in which there are ≥ 20% basophils in the blood (Arber et al., 2016). These basophils are often immature and express some CD117 (Valent et al., 2018). One study showed that immature CML basophils express and release tryptase (Sperr et al., 2014). Currently basophils and mast cells are distinguished based on surface CD117 expression and intracellular tryptase. Since tryptase is mainly a mast cell marker and we detected circulating mast cells in CML patients, it raises questions on the actual identity of the CD117-expressing basophils described in CML. It is interesting to speculate that there is a disturbed differentiation of a shared precursor of mast cells and basophils in these patients resulting in the $FcεRI^+$ $CD117^+$ cells, however this needs to be further investigated.

Based on the differentiation experiments we designed a new culture protocol to study the mast cell potential, using only IL-3 and IL-6 for the first 5 days in the culture. In this way we promote mast cell progenitor survival and differentiation without creating bias towards the mast cell lineage. However, SCF will is required for complete maturation of pre-mast cells.

4.3 *KIT* D816V IN SINGLE CELLS (STUDY III)

The *KIT* D816V mutation is present in different lineages and detected in bulk sorted $CD34^+$ progenitors. In this study we aimed to identify the origin of the *KIT* D816V mutation in the hematopoietic landscape, using bone marrow samples from patients under evaluation for systemic mastocytosis.

For this study we designed a multi-color flow cytometry panel that could identify different progenitor populations as well as mature mast cells (**Figure 1 in study III**). Analysis of the percentage of $CD34^+$ HSPCs did not give a skewed hematopoiesis in SM, commonly observed in myeloid neoplasms, and shown in the hematopoietic profile for the two MDS/MPN patients included in this study. There was no difference in the total percentage of $CD34^+$ cells for any SM subtype (**Supplementary figure 2 in study III**). We observed a trend towards a lower percentage of lymphoid progenitors, the BNKP, in patients compared to no SM controls, which is expected since the production of lymphoid cells declines with increasing age and the control subjects were slightly younger. In addition, colony forming potential of CMPs, GMPs and MEPs was similar in SM and no SM subjects.

Detailed analysis of the flow cytometry data of all patients revealed aberrant immunophenotype of CD45RA on mast cells of SM patients only (**Figure 3 in study III**). Interestingly, one control subject was diagnosed with CM and had slightly higher CD45RA expression. It will be interesting to follow this patient and assess whether this patients progresses into SM. One SM patient had lower CD45RA expression, which can be explained by the diagnosis. The biopsy at the time of the aspirate did not show atypical morphology of mast cells, thereby not giving three minor criteria to be diagnosed with SM. However, a consecutive biopsy showed atypical morphology, giving the diagnosis of ISM. Aberrant CD45RA expression in SM has not been described before and there are no reported explanations for aberrant CD45RA expression. However, CD45RA expression is associated with IL-6, one of the elevated mast cell mediators in SM patients (Zheng et al., 2015).

We identified a subpopulation in the CMP that expresses FcεRI, with similar expression to the circulating MCP (**Supplementary figure 3 in study III**). We therefore hypothesized that this subpopulation harbored mast cell potential. In vitro cultures showed high mast cell potential in the $CMP^{Fc\varepsilon RI+}$, which we did not observe in $CMPs^{Fc\varepsilon RI-}$ and GMPs (**Figure 2 and supplementary figure 5 in study III**). Additional sorts of subpopulations of the $CMP^{Fc\varepsilon RI+}$ indicated that the mast cell potential different comparing $CD203c^+$ and $CD203c^-$ cells. These findings were further assessed in study IV.

As mentioned, the main aim of this study was to identify the *KIT* D816V mutation in hematopoiesis. We developed a highly sensitive method to detect the mutation in single cells (**Supplementary figure 6 in study III**). We found that the majority of mast cells in SM patients

are mutated, with similar percentages in AdvSM compared to ISM (**Figure 4 in study III**). Interestingly we found that the *KIT* D816V mutated mast cells had higher CD45RA expression compared to mast cells that had normal *KIT*. As mentioned before, *KIT* D816V has been detected in multiple lineages, therefore we hypothesized that the mutation is present in multipotent progenitors and that it is enriched in the mast cell lineage. Indeed we found the mutation throughout the hematopoietic landscape; however, we did not find a higher percentage of mutated cells in the $CMP^{FcεRI+}$ population (**Figure 4F in study III**). In addition we sorted MCPs from blood and found a similar mutation rate in these cells compared to bone marrow HSPCs in one patient (SM16). Thus, the high mutation rate in mature mast cells was not reflected in any progenitor population. Instead, we found the *KIT* D816V mutation in HSCs, which helps our understanding about the onset of SM.

It is estimated that about 80% of the SM patients carry *KIT* D816V (Valent et al., 2017). However, this assumption is based on mutation analysis of DNA isolated from whole blood and bone marrow or from bulk-sorted cells. It was reported that 76/105 (72%) ISM patients carried *KIT* D816V in bulk sorted $CD34^+$ cells (Jara-Acevedo et al., 2015). With our highly sensitive method we were able to find only a few mutated cells from the in total more than 1000 single sorted cells. Using DNA from sorted $CD34^+$ cells, these patients would likely have been identified as negative for *KIT* D816V in progenitors. Therefore it is tempting to speculate that all SM patients carry the *KIT* D816V mutation, but present methodology is unable to detect this, however it is currently also not feasible to do single cell DNA analysis on all SM patients.

So when is the *KIT* D816V mutation first acquired? We know that it is not a germline mutation, so it is likely acquired spontaneously at a certain stage of development. One possibility is that there is one long-term HSC that acquires the *KIT* D816V mutation and produces daughter cells with the *KIT* D816V mutation during a lifetime, resulting in an increased number of mutated mast cells with increased age. Another option is that the mutation is acquired in an early ancestor cell that gives hematopoietic cells and mesenchymal cells. This theory is supported by the previously published data that mesenchymal stromal cells express *KIT* D816V (Garcia-Montero et al., 2016; Nemeth et al., 2015). However, it remains to be confirmed whether the mutation is acquired in a common ancestor or separately in hematopoietic and mesenchymal cell lineages. The existence of a common ancestor cell for hematopoietic and mesenchymal cells has not been shown so far, which makes it unlikely that the *KIT* D816V mutation in both lineages arises from the same clone. Also, as mentioned in the introduction of this thesis, hematopoietic stem cells arise from aorta-gonad-mesonephros region or in earlier stages from the extra-embryonic yolk sac. If this hypothetical progenitor of HSCs and mesenchymal stem cells exists, it needs to be a cell at embryonic development.

KIT is expressed in progenitors, but not required for MCP survival, as we showed in study II. In SM, *KIT* D816V affects mast cells shown by their aberrant phenotype and high mutation rate. In contrast, the low mutation burden in MCPs suggests that the *KIT* D816V mutation does not affect differentiation of HSPCs and their function. Several studies reported multi-lineage

involvement of *KIT* D816V, as we confirmed in different HSPCs, but so far there are no studies showing aberrant function of other cells than mast cells in SM.

Altogether, this study provides novel insights on the origin of the *KIT* D816V mutation in stem and progenitor cells of SM whereas the overall hematopoietic profile is unaffected. We also identified CD45RA as an aberrant marker specific for SM mast cells, which can be used as an additional marker in diagnosis of patients. Furthermore, the observed enriched mast cell potential in the FcεRI^{+} CMPs provides new clues on mast cell origin in human bone marrow.

4.4 MAST CELL AND BASOPHIL PROGENITORS (STUDY IV)

The bone marrow from mice contains unipotent and bipotent mast cell progenitors, however, the existence of similar progenitors in the human bone marrow is yet to be confirmed. In study III we described a bone marrow progenitor with mast cell potential that expressed FcεRI, which we aimed to further characterize in this study.

We distinguished subpopulations within the CMP$^{FcεRI+}$ population using the markers CD203c and Integrin β7 (**Figure 1 in study IV**). In vitro cultures showed that CD203c^{+} progenitors only gave basophils and mast cells, whereas CD203c^{-} progenitors exhibited the potential to differentiate into erythroid cells, as well as basophils and mast cells. (**Figure 3 and 4 in study IV**).

The lack of erythroid potential in CD203c^{+} CMPs$^{FcεRI+}$ could be predicted by the presence of CD33 surface expression, since this marker is known to be downregulated during erythroid differentiation (**Figure 2 in study IV**). CD33 is a marker expressed on aberrant mast cells in a large cohort of patients, with the highest expression in ISM and well-differentiated SM (Dasilva-Freire et al., 2019). It remains to be investigated whether CD33 expression is affected in the mature mast cells of the mastocytosis patients included in this study.

As mentioned in the introduction, several studies present data that argues for a shared differentiation trajectory for basophils, eosinophils, and mast cells. Culture experiments showed that eosinophils develop from an IL-5R^{+} subpopulation of the CMP (Görgens et al., 2013; Mori et al., 2009). Mature eosinophils express CD203c and Iβ7, like basophils, but lack FcεRI. Common gating strategies use a combination of CD16 and CD15 to distinguish between neutrophils and eosinophils, CD16^{+} CD15^{-} versus CD16^{+} CD15^{+} respectively. It is possible that the fraction of CD15^{+} expressing cells in CMP cultures represents eosinophils (**Figure 3 and 4 in study IV**). However, in vitro cultures of human pluripotent stem cells in medium supplemented with IL-3 and IL-5 showed that eosinophils did not express CD15 after 4-5 days (Choi et al., 2011). In addition, the CD15^{+} population from cultured CMPs$^{FcεRI-}$ was phenotypically similar to the CD15^{+} cells from cultured GMPs, which makes it more likely that they represent a pre-monocyte-neutrophil cell.

Our culture system did not assess the megakaryocyte potential. As mentioned in the introduction of this thesis, classical hematopoiesis models describe a shared megakaryocyte and erythroid progenitor that arises from the CMP and MEP. More recent studies suggest that megakaryocytes can develop directly from the HSC. Since some CMPs$^{FcεRI+}$ progenitors in our

model still have erythroid potential, it will be interesting to assess whether these cells have any megakaryocyte potential. The difficulty with analyzing cell-potential with culture medium that stimulates all lineages is that hematopoietic cells differentiate in different growth rates; for example, about 99% of all the blood cellular components consist of erythrocytes and platelets. Thus, whereas a culture systems with only limited growth factors might give a limited differentiation and create biased data, addition of multi-lineage stimulating growth factors results in a more realistic differentiation with mainly erythrocytes and platelets, in which rare populations are difficult to detect. Therefore, in this study we cultured cells in limited medium conditions to identify the basophil-mast cell potential as well as multilineage medium conditions to assess the potency to form other cells.

In this study we measured cell-potential by flow cytometry analysis, whereas there are other methods to define the cell composition, such as immunohistochemistry or RNA-expression analysis. Flow cytometry analysis was our preferred method since it enabled us to assess the expression of multiple cell surface receptors in one panel of limited cell numbers, including a live-dead marker to only compare expression in living cells. We included other markers in the panel, such as CD123 (IL-3R), siglec-8, and CD11b, however these marker were not expressed or did not give sufficient staining to draw conclusions on their expression. In future experiment it would be interesting to include other markers, such as CXCR3, to identify basophils and eosinophils, and confirm the identity of cultured cells by morphology staining and RNA-expression analysis.

Taken together, this study provides novel insights in the cell-forming potential of a subset of CMPs. We show a clear separation in differentiation of myeloid cells in our culture experiments. It is interesting to speculate why mast cells, basophils and likely eosinophils are closely related to the erythroid and megakaryocyte lineage, especially since their morphology and function is more similar to other granulocytes.

5 CONCLUDING REMARKS AND OUTLOOK

We aimed to gain more insight in a rare cell type and a rare disease, creating challenging projects. The expertise in our lab and available methods, together with a great collaboration with clinicians, made it possible to study mast cell development. However, there are still several open questions on basic mast cell development and the role of mast cells in systemic mastocytosis, providing a starting point for further research.

Combining the data in these studies, we revealed new concepts on mast cell development: their close relation to the erythroid trajectory and not to neutrophils, as well as the role of SCF and KIT-signaling in initial mast cell differentiation. Given that KIT-signaling is not required, what factors drive mast cell differentiation? Signaling through the IL-3R resulted in MCP survival and proliferation, whereas IL-3 is mainly known for basophil maturation, so which other factors drive mast cells differentiation, or is basophil and mast cell development shared until they leave the bone marrow as MCP or basophil? And if we go a step back in hierarchy, what separates mast cell, basophil, and eosinophil from neutrophil development?

The finding that KIT-signaling is not required in early mast cell development explains why the *KIT* D816V mutation does not affect the hematopoiesis landscape in SM patients. As a consequence, the effects of this mutation is only noticed when mast cells mature and require KIT-signaling for their normal function. Recently a leukemic initiating cell population was reported in MCL and it would be interesting to assess whether these cells express *KIT* D816V and if this is involved in the development of mastocytosis.

Currently there is still limited knowledge on the role of *KIT* D816V in disease ontology; what causes the *KIT* D816V mutation and is this mutation the driver in SM? If it is not in the germline, where does it first arise? Does is arise spontaneously? On the other hand, the D816V mutation is not the only mutation found in the KIT receptor, as there have been more than 500 reported *KIT* mutations. So, what makes this receptor so vulnerable to genetic alterations? In this thesis we studied the *KIT* D816V in relation to the mast cell lineage, but what is the effect of *KIT* D816V on the development of other cell lineages? And what is the prevalence and role of other associated mutations in these *KIT* D816V mutated cells? The development of high-throughput single cell genome sequencing techniques will be very useful for answering these questions, but these techniques are still in the developmental stage. It will be very interesting to create a genetic footprint of the patients that allows for better and personalized treatment, as is currently seen for other myeloid neoplasms.

Besides the presence of *KIT* D816V throughout the hematopoietic landscape, we present other novel findings on SM ontology: SM-specific CD45RA expressing mast cells and the presence of circulating mast cells in advanced SM. These findings are relevant for basic understanding of SM, but can also be applied in the clinic. However, implementation of findings in translational research is challenging. For example, in order to be implemented as diagnostic criterion or in prognostic research, our findings need to be confirmed in a larger cohort of patients. This may prove to be more difficult than expected, as this is usually not of interest of fundamental scientists, and clinicians might not see the importance of this either. Thus, a challenge for the

future is to further improve collaborations between scientists and clinicians especially for a rare disease like SM. A good example in which this is pursued is the European Competence Network on Mastocytosis, a network with fundamental scientists and clinicians. Within this network, clinicians and scientists share knowledge on SM and aim for improved recognition, diagnosis, and therapy. In addition, most countries within Europe have a center of excellence or a reference center consisting of local scientist and clinicians who focus on SM and can provide advice to hospitals or universities.

In conclusion, the research conducted in this thesis provides novel insights and on mast cell development in health and systemic mastocytosis, which in the end will hopefully be beneficial for the patients suffering from mast cell activation diseases.

6 POPULAR SCIENCE SUMMARY

All white blood cells origin from a stem cell, called the hematopoietic stem cell. This hematopoietic stem cell is located in the bone marrow and forms supplies your body with blood cells, including white blood cells, red blood cells and platelets, during your whole life. Mast cells are a type of white blood cell, which detect stranger of danger signals that are potentially harmful. Some white blood cells circulate in the blood, others, like the mast cell are living in tissues or organs.

When a stem cell develops into a mature white blood cell, it starts to change the DNA expression inside the cell, which in turn results into changes in the receptors (signal receivers) on the surface of the cell. Before a stem cell transforms into a mast cell, it first becomes a mast cell precursor that leaves the bone marrow and enters the blood stream. When this mast cell precursor arrives at its destination, it moves into the tissue and matures. There are several different methods available nowadays to detect these changes on the surface and inside the cell. We can identify mast cells from other white blood cells by their specific receptors on the surface, and by the DNA expression in the cell. For example, mature mast cells have a lot of the receptors called KIT and FcεRI. We can label these receptors with a fluorescent label and detect them using a flow cytometer. In total we can detect up to 16 different receptors on one cell, making it possible to detect all different kinds of white blood cells in a blood or bone marrow sample. Using this method we can also identify new cells, or new receptors on an already known cell. This method is well established in this research field and therefore used in all four studies that are the basis of this thesis.

The overall aim of this thesis project was to get a better understanding on normal mast cell development and mast cell development in a disease called systemic mastocytosis. In this disease, most of the mast cells have a mutation in the receptor KIT called D816V. This mutation affects the function of mast cells, which in most cases results in an increase of these mast cells in the skin (presented as typical brown-red spots) or in the bone marrow, the liver, the spleen, or the intestine. Patients that have a mild form of the disease have symptoms such as hives, itching, and gastrointestinal problems. When the disease is aggressive, the mast cells start damaging the tissues or organs that they infiltrated. Patients living with the mild form have a normal life expectancy, whereas patients with the aggressive disease have a bad prognosis of only a few months to years. At the moment there is no cure and patients mainly take symptom-reducing medication.

This thesis contains four studies that will help to answer the aim of the thesis:

In study I we analyzed blood samples from patients with different forms of mastocytosis. We found that patients with the aggressive form of the disease have mature mast cells in the blood, which are absent in the mild form and healthy persons.

In study II we investigated what signals are necessary for the development of mast cells. We found that the receptor KIT is important for the mast cell to get fully mature, but not for the initial development from the stem cell.

In study III we developed a method that detects the D816V mutation on the receptor KIT in single cells from patients to identify where this mutation comes from. We could detect the mutation in a small percentage of the stem cells from the patients. In contrast, almost all mast cells in the same patient have the mutation. Further analysis of the bone marrow cells with flow cytometry showed that the mast cells in the patients have an abnormal receptor on their cell surface, called CD45RA. In addition, we found a rare cell in the bone marrow that developed into mast cells when we isolated this cell and cultured it in the lab.

In study IV we continued to analyze the rare mast cell precursors that we identified in study III. By isolating developing cells and culturing them in the lab, we found that mast cells development is closely related to the development of another white blood cell, the basophil.

To sum up, the data in the studies of this thesis help us to get a better basic understanding of mast cell development in healthy persons and in patients with systemic mastocytosis. The findings in all four studies are useful for the field of basic mast cell research and for clinical research in which we aim to identify new targets to treat mastocytosis patients.

7 POPULAIR WETENSCHAPPELIJKE SAMENVATTING

Alle witte bloedcellen komen van een stamcel die we de hematopoëtische stamcel noemen. Deze hematopoëtische stamcel bevindt zich in het beenmerg en vormt gedurende je hele leven alle soorten bloedcellen, zoals witte bloedcellen, rode bloedcellen en bloedplaatjes. Mestcellen zijn een soort witte bloedcel die in je lichaam vreemde en gevaarlijke signalen kunnen herkennen die potentieel schadelijk voor je zijn. Sommige witte bloedcellen circuleren in het bloed, anderen, zoals de mestcel, leven in weefsels of in organen.

Als een stamcel zich tot een mestcel ontwikkelt verandert de expressie van het DNA in de cel en de expressie van receptoren (signaalontvangers) die op de buitenkant van de cel zitten. Voordat een stamcel zich tot een volwassen mestcel ontwikkelt, verlaat de cel het beenmerg als voorloper mestcel en gaat het de bloedstroom in. Zodra de voorloper mestcel aankomt op zijn bestemming, verlaat deze het bloed, migreert naar het weefsel, en rijpt verder tot volwassen mestcel. Er zijn tegenwoordig verschillende methoden beschikbaar om deze veranderingen in de cel en op de buitenkant van de cel te meten. Zo kunnen we mestcellen van andere witte bloedcellen onderscheiden door hun specifieke DNA expressie en specifieke receptoren. Volwassen mestcellen hebben bijvoorbeeld veel van de receptoren KIT en FcεRI. We kunnen deze receptoren labelen met een fluorescerend label en dat vervolgens meten met een flow cytometer. Met deze methode kunnen we in totaal 16 verschillende receptoren meten op een enkele cel, wat het mogelijk maakt om verschillende witte bloedcellen in het bloed en in het beenmerg te identificeren. Met deze methode kunnen we ook nieuwe cellen of nieuwe receptoren identificeren. Deze methode is ver ontwikkeld in dit onderzoeksgebied en is daarom ook de basis voor alle vier de studies in dit proefschrift.

Het doel van dit proefschrift is om een beter beeld te krijgen van de normale ontwikkeling van de mestcel in vergelijking met de ontwikkeling van de mestcel in de ziekte systematische mastocytose. Deze ziekte wordt gekenmerkt door de aanwezigheid van een mutatie in de receptor KIT, genaamd D816V. De mutatie heeft invloed op de functie van mestcellen waardoor deze mestcellen toenemen in de huid (waargenomen als typische bruinrode vlekjes) of in het beenmerg, de lever, de milt of de darmen. Patiënten met een milde vorm van mastocytose hebben symptomen zoals opvliegers, jeuk of darmproblemen. Wanneer er sprake is van een agressieve vorm, kunnen de mestcellen schade toebrengen aan de weefsels en organen die ze hebben geïnfiltreerd. Patiënten met een milde vorm hebben een normale levensverwachting, maar patiënten met een agressieve vorm hebben een slechte prognose van een paar maanden tot jaren. Op dit moment is mastocytose niet te genezen en behandelingen zijn daardoor gericht op het bestrijden van de symptomen.

Dit proefschrift is gebaseerd op vier studies:

In studie I bestudeerden we bloed van patiënten met verschillende vormen van mastocytose. De resultaten toonden aan dat patiënten met een agressieve vorm van mastocytose volwassen mestcellen hebben in het bloed, maar dat deze afwezig zijn in patiënten met een milde vorm en in gezonde personen.

In studie II onderzochten we welke signalen belangrijk zijn voor de ontwikkeling van mestcellen. Celkweek experimenten lieten zien dat de receptor KIT niet belangrijk is voor de initiële ontwikkeling van de stamcel tot mestcel, maar wel voor de uiteindelijke rijping van de mestcel.

In studie III ontwikkelden we een methode om de mutatie D816V in de receptor KIT in een enkele cel te kunnen detecteren om vervolgens de oorsprong van deze mutatie in voorlopercellen vast te stellen. De mutatie was aanwezig in een deel van de stamcellen van de patiënten en in bijna alle volwassen mestcellen van dezelfde patiënt. Door verdere analyse van het beenmerg met de flow cytometer ontdekten we een nieuw signaalmolecuul dat alleen aanwezig is op mestcellen van patiënten, genaamd CD45RA. Verder hebben we ook een zeldzame voorlopercel in het beenmerg gevonden die zich in kweek tot mestcel ontwikkelt.

In studie IV gingen we verder met het analyseren van de zeldzame voorlopercel in het beenmerg die we in studie III hadden gevonden. Door deze voorlopercellen te isoleren en in het lab te kweken vonden we dat de ontwikkeling van mestcellen nauw is verbonden met de ontwikkeling van een andere witte bloedcel, de basofiel.

Samengevat, de resultaten in dit proefschrift zijn belangrijk om een beter beeld te krijgen over de ontwikkeling van mestcellen in gezonde personen en patiënten met mastocytose. De bevindingen hebben toegevoegde waarde in fundamenteel onderzoek naar mestcellen, maar ook voor klinisch onderzoek dat gericht is op het ontwikkelen van een betere behandeling voor mastocytose patiënten.

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9 REFERENCES

Abraham, S.N., and St John, A.L. (2010). Mast cell-orchestrated immunity to pathogens. Nat Rev Immunol 10(6), 440-452.

Afonja, O., Amorosi, E., Ashman, L., and Takeshita, K. (1998). Multilineage involvement and erythropoietin-"independent" erythroid progenitor cells in a patient with systemic mastocytosis. Ann Hematol 77(4), 183-186.

Alvarez-Twose, I., Matito, A., Morgado, J.M., Sanchez-Munoz, L., Jara-Acevedo, M., Garcia-Montero, A., Mayado, A., Caldas, C., Teodosio, C., Munoz-Gonzalez, J.I., *et al.* (2017). Imatinib in systemic mastocytosis: a phase IV clinical trial in patients lacking exon 17 KIT mutations and review of the literature. Oncotarget 8(40), 68950-68963.

Arber, D.A., Orazi, A., Hasserjian, R., Thiele, J., Borowitz, M.J., Le Beau, M.M., Bloomfield, C.D., Cazzola, M., and Vardiman, J.W. (2016). The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood 127(20), 2391-2405.

Arinobu, Y., Iwasaki, H., Gurish, M.F., Mizuno, S., Shigematsu, H., Ozawa, H., Tenen, D.G., Austen, K.F., and Akashi, K. (2005). Developmental checkpoints of the basophil/mast cell lineages in adult murine hematopoiesis. Proceedings of the National Academy of Sciences of the United States of America 102(50), 18105-18110.

Arock, M., Sotlar, K., Akin, C., Broesby-Olsen, S., Hoermann, G., Escribano, L., Kristensen, T.K., Kluin-Nelemans, H.C., Hermine, O., Dubreuil, P., *et al.* (2015). KIT mutation analysis in mast cell neoplasms: recommendations of the European Competence Network on Mastocytosis. Leukemia 29(6), 1223-1232.

Balci, T.B., Prykhozhij, S.V., Teh, E.M., Da'as, S.I., McBride, E., Liwski, R., Chute, I.C., Leger, D., Lewis, S.M., and Berman, J.N. (2014). A transgenic zebrafish model expressing KIT-D816V recapitulates features of aggressive systemic mastocytosis. British Journal of Haematology 167(1), 48-61.

Baumgartner, C., Cerny-Reiterer, S., Sonneck, K., Mayerhofer, M., Gleixner, K.V., Fritz, R., Kerenyi, M., Boudot, C., Gouilleux, F., Kornfeld, J.W., *et al.* (2009). Expression of activated STAT5 in neoplastic mast cells in systemic mastocytosis: subcellular distribution and role of the transforming oncoprotein KIT D816V. Am J Pathol 175(6), 2416-2429.

Bibi, S., Zhang, Y., Hugonin, C., Mangean, M.D., He, L., Wedeh, G., Launay, J.M., Van Rijn, S., Wurdinger, T., Louache, F., *et al.* (2016). A new humanized in vivo model of KIT D816V+ advanced systemic mastocytosis monitored using a secreted luciferase. Oncotarget 7(50), 82985-83000.

Bischoff, S.C., and Dahinden, C.A. (1992). c-kit ligand: a unique potentiator of mediator release by human lung mast cells. J Exp Med 175(1), 237-244.

Bodemer, C., Hermine, O., Palmerini, F., Yang, Y., Grandpeix-Guyodo, C., Leventhal, P.S., Hadj-Rabia, S., Nasca, L., Georgin-Lavialle, S., Cohen-Akenine, A., *et al.* (2009). Pediatric Mastocytosis Is a Clonal Disease Associated with D816V and Other Activating c-KIT Mutations. J Invest Dermatol 130(3), 804-815.

Borate, U., Mehta, A., Reddy, V., Tsai, M., Josephson, N., and Schnadig, I. (2016). Treatment of CD30-positive systemic mastocytosis with brentuximab vedotin. Leuk Res 4425-31.

Boyce, J.A., Friend, D., Matsumoto, R., Austen, K.F., and Owen, W.F. (1995). Differentiation in vitro of hybrid eosinophil/basophil granulocytes: autocrine function of an eosinophil developmental intermediate. J Exp Med 182(1), 49-57.

Brockow, K., Akin, C., Huber, M., Scott, L.M., Schwartz, L.B., and Metcalfe, D.D. (2002). Levels of mast-cell growth factors in plasma and in suction skin blister fluid in adults with mastocytosis: correlation with dermal mast-cell numbers and mast-cell tryptase. J Allergy Clin Immunol 109(1), 82-88.

Buhring, H.J., Simmons, P.J., Pudney, M., Muller, R., Jarrossay, D., van Agthoven, A., Willheim, M., Brugger, W., Valent, P., and Kanz, L. (1999). The monoclonal antibody 97A6 defines a novel surface antigen expressed on human basophils and their multipotent and unipotent progenitors. Blood 94(7), 2343-2356.

Butterfield, J.H., Ravi, A., and Pongdee, T. (2018). Mast Cell Mediators of Significance in Clinical Practice in Mastocytosis. Immunol Allergy Clin North Am 38(3), 397-410.

Carroll-Portillo, A., Cannon, J.L., te Riet, J., Holmes, A., Kawakami, Y., Kawakami, T., Cambi, A., and Lidke, D.S. (2015). Mast cells and dendritic cells form synapses that facilitate antigen transfer for T cell activation. The Journal of cell biology 210(5), 851-864.

Chen, C.C., Grimbaldeston, M.A., Tsai, M., Weissman, I.L., and Galli, S.J. (2005). Identification of mast cell progenitors in adult mice. Proceedings of the National Academy of Sciences of the United States of America 102(32), 11408-11413.

Cherian, S., McCullouch, V., Miller, V., Dougherty, K., Fromm, J.R., and Wood, B.L. (2016). Expression of CD2 and CD25 on mast cell populations can be seen outside the setting of systemic mastocytosis. Cytometry B Clin Cytom 90(4), 387-392.

Chian, R., Young, S., Danilkovitch-Miagkova, A., Ronnstrand, L., Leonard, E., Ferrao, P., Ashman, L., and Linnekin, D. (2001). Phosphatidylinositol 3 kinase contributes to the transformation of hematopoietic cells by the D816V c-Kit mutant. Blood 98(5), 1365-1373.

Chisholm, K.M., Merker, J.D., Gotlib, J.R., Gitana, G., Lefterova, M., Zehnder, J.L., George, T.I., Arber, D.A., and Ohgami, R.S. (2015). Mast cells in systemic mastocytosis have distinctly brighter CD45 expression by flow cytometry. Am J Clin Pathol 143(4), 527-534.

Choi, K.D., Vodyanik, M., and Slukvin, II (2011). Hematopoietic differentiation and production of mature myeloid cells from human pluripotent stem cells. Nat Protoc 6(3), 296-313.

Civin, C.I., Strauss, L.C., Brovall, C., Fackler, M.J., Schwartz, J.F., and Shaper, J.H. (1984). Antigenic analysis of hematopoiesis. III. A hematopoietic progenitor cell surface antigen defined by a monoclonal antibody raised against KG-1a cells. J Immunol 133(1), 157-165.

Clark-Brelje, T., and Sorenson, R.L. (2005-2019). Histology Guide. In Chapter 7 - Peripheral blood basophil (histologyguide.org/EM-atlas/07-peripheral-blood.html).

Cohen, S.S., Skovbo, S., Vestergaard, H., Kristensen, T., Moller, M., Bindslev-Jensen, C., Fryzek, J.P., and Broesby-Olsen, S. (2014). Epidemiology of systemic mastocytosis in Denmark. Br J Haematol 166(4), 521-528.

D'Ambrosio, C., Akin, C., Wu, Y., Magnusson, M.K., and Metcalfe, D.D. (2003). Gene expression analysis in mastocytosis reveals a highly consistent profile with candidate molecular markers. J Allergy Clin Immunol 112(6), 1162-1170.

Dahl, C., Hoffmann, H.J., Saito, H., and Schiotz, P.O. (2004). Human mast cells express receptors for IL-3, IL-5 and GM-CSF; a partial map of receptors on human mast cells cultured in vitro. Allergy 59(10), 1087-1096.

Dahlin, J.S., Hamey, F.K., Pijuan-Sala, B., Shepherd, M., Lau, W.W.Y., Nestorowa, S., Weinreb, C., Wolock, S., Hannah, R., Diamanti, E., *et al.* (2018). A single-cell hematopoietic landscape resolves 8 lineage trajectories and defects in Kit mutant mice. Blood 131(21), e1-e11.

Dahlin, J.S., Malinovschi, A., Ohrvik, H., Sandelin, M., Janson, C., Alving, K., and Hallgren, J. (2016). Lin- CD34hi CD117int/hi FcepsilonRI+ cells in human blood constitute a rare population of mast cell progenitors. Blood 127(4), 383-391.

Dasilva-Freire, N., Mayado, A., Teodosio, C., Jara-Acevedo, M., Alvarez-Twose, I., Matito, A., Sanchez-Munoz, L., Caldas, C., Henriques, A., Munoz-Gonzalez, J.I., *et al.* (2019). Bone Marrow Mast Cell Antibody-Targetable Cell Surface Protein Expression Profiles in Systemic Mastocytosis. Int J Mol Sci 20(3), E552.

Dastych, J., and Metcalfe, D.D. (1994). Stem cell factor induces mast cell adhesion to fibronectin. J Immunol 152(1), 213-219.

de Barros, C.M., Andrade, L.R., Allodi, S., Viskov, C., Mourier, P.A., Cavalcante, M.C., Straus, A.H., Takahashi, H.K., Pomin, V.H., Carvalho, V.F., *et al.* (2007). The Hemolymph of the ascidian Styela plicata (Chordata-Tunicata) contains heparin inside basophil-like cells and a unique sulfated galactoglucan in the plasma. J Biol Chem 282(3), 1615-1626.

De Vita, S., Schneider, R.K., Garcia, M., Wood, J., Gavillet, M., Ebert, B.L., Gerbaulet, A., Roers, A., Levine, R.L., Mullally, A., *et al.* (2014). Loss of function of TET2 cooperates with constitutively active KIT in murine and human models of mastocytosis. PLoS One 9(5), e96209.

Denburg, J.A., Telizyn, S., Messner, H., Lim, B., Jamal, N., Ackerman, S.J., Gleich, G.J., and Bienenstock, J. (1985). Heterogeneity of human peripheral blood eosinophil-type colonies: evidence for a common basophil-eosinophil progenitor. Blood 66(2), 312-318.

Diaz-Agustin, B., Escribano, L., Bravo, P., Herrero, S., Nunez, R., Navalon, R., Navarro, L., Torrelo, A., Cantalapiedra, A., del Castillo, L., *et al.* (1999). The CD69 early activation molecule is overexpressed in human bone marrow mast cells from adults with indolent systemic mast cell disease. British Journal of Haematology 106(2), 400-405.

Doulatov, S., Notta, F., Eppert, K., Nguyen, L.T., Ohashi, P.S., and Dick, J.E. (2010). Revised map of the human progenitor hierarchy shows the origin of macrophages and dendritic cells in early lymphoid development. Nat Immunol 11(7), 585-593.

Dowse, R., Ibrahim, M., McLornan, D.P., Moonim, M.T., Harrison, C.N., and Radia, D.H. (2017). Beneficial effects of JAK inhibitor therapy in Systemic Mastocytosis. Br J Haematol 176(2), 324-327.

Druker, B.J., Talpaz, M., Resta, D.J., Peng, B., Buchdunger, E., Ford, J.M., Lydon, N.B., Kantarjian, H., Capdeville, R., Ohno-Jones, S., *et al.* (2001). Efficacy and safety of a specific inhibitor of the BCR-ABL tyrosine kinase in chronic myeloid leukemia. N Engl J Med 344(14), 1031-1037.

Dwyer, D.F., Barrett, N.A., Austen, K.F., and Immunological Genome Project, C. (2016). Expression profiling of constitutive mast cells reveals a unique identity within the immune system. Nat Immunol 17(7), 878-887.

Eisenwort, G., Sadovnik, I., Schwaab, J., Jawhar, M., Keller, A., Stefanzl, G., Berger, D., Blatt, K., Hoermann, G., Bilban, M., *et al.* (2019). Identification of a leukemia-initiating stem cell in human mast cell leukemia. Leukemia.

Escribano, L., Diaz-Agustin, B., Bellas, C., Navalon, R., Nunez, R., Sperr, W.R., Schernthaner, G.H., Valent, P., and Orfao, A. (2001). Utility of flow cytometric analysis of mast cells in the diagnosis and classification of adult mastocytosis. Leuk Res 25(7), 563-570.

Escribano, L., Orfao, A., Agustin, B.D., Cervero, C., Herrero, S., Villarrubia, J., Bravo, P., Torrelo, A., Montero, T., Valdemoro, M., *et al.* (1998a). Human bone marrow mast cells from indolent systemic mast cell disease constitutively express increased amounts of the CD63 protein on their surface. Cytometry 34(5), 223-228.

Escribano, L., Orfao, A., Diaz-Agustin, B., Villarrubia, J., Cervero, C., Lopez, A., Marcos, M.A., Bellas, C., Fernandez-Canadas, S., Cuevas, M., *et al.* (1998b). Indolent systemic mast cell disease in adults: immunophenotypic characterization of bone marrow mast cells and its diagnostic implications. Blood 91(8), 2731-2736.

Florian, S., Sonneck, K., Hauswirth, A.W., Krauth, M.T., Schernthaner, G.H., Sperr, W.R., and Valent, P. (2006). Detection of molecular targets on the surface of CD34+/CD38-- stem cells in various myeloid malignancies. Leuk Lymphoma 47(2), 207-222.

Födinger, M., Fritsch, G., Winkler, K., Emminger, W., Mitterbauer, G., Gadner, H., Valent, P., and Mannhalter, C. (1994). Origin of human mast cells: development from transplanted hematopoietic stem cells after allogeneic bone marrow transplantation. Blood 84(9), 2954-2959.

Frankel, A.E., Woo, J.H., Ahn, C., Pemmaraju, N., Medeiros, B.C., Carraway, H.E., Frankfurt, O., Forman, S.J., Yang, X.A., Konopleva, M., *et al.* (2014). Activity of SL-401, a targeted therapy directed to interleukin-3 receptor, in blastic plasmacytoid dendritic cell neoplasm patients. Blood 124(3), 385-392.

Friesen, C.A., Neilan, N., Daniel, J.F., Radford, K., Schurman, J.V., Li, D.Y., Andre, L., St Peter, S.D., and Holcomb, G.W., 3rd (2011). Mast cell activation and clinical outcome in pediatric cholelithiasis and biliary dyskinesia. BMC Res Notes 4(1), 322.

Furitsu, T., Tsujimura, T., Tono, T., Ikeda, H., Kitayama, H., Koshimizu, U., Sugahara, H., Butterfield, J.H., Ashman, L.K., Kanayama, Y., *et al.* (1993). Identification of mutations in the coding sequence of the proto-oncogene c-kit in a human mast cell leukemia cell line causing ligand-independent activation of c-kit product. The Journal of clinical investigation 92(4), 1736-1744.

Gabillot-Carre, M., Lepelletier, Y., Humbert, M., de Sepuvelda, P., Hamouda, N.B., Zappulla, J.P., Liblau, R., Ribadeau-Dumas, A., Machavoine, F., Letard, S., *et al.* (2006). Rapamycin inhibits growth and survival of D816V-mutated c-kit mast cells. Blood 108(3), 1065-1072.

Garcia-Montero, A.C., Jara-Acevedo, M., Alvarez-Twose, I., Teodosio, C., Sanchez-Munoz, L., Muniz, C., Munoz-Gonzalez, J.I., Mayado, A., Matito, A., Caldas, C., *et al.* (2016). KIT D816V-mutated bone marrow mesenchymal stem cells in indolent systemic mastocytosis are associated with disease progression. Blood 127(6), 761-768.

Garcia-Montero, A.C., Jara-Acevedo, M., Teodosio, C., Sanchez, M.L., Nunez, R., Prados, A., Aldanondo, I., Sanchez, L., Dominguez, M., Botana, L.M., *et al.* (2006). KIT mutation in mast cells and other bone marrow hematopoietic cell lineages in systemic mast cell disorders: a prospective study of the Spanish Network on Mastocytosis (REMA) in a series of 113 patients. Blood 108(7), 2366-2372.

Gentek, R., Ghigo, C., Hoeffel, G., Bulle, M.J., Msallam, R., Gautier, G., Launay, P., Chen, J., Ginhoux, F., and Bajenoff, M. (2018). Hemogenic Endothelial Fate Mapping Reveals Dual Developmental Origin of Mast Cells. Immunity 48(6), 1160-1171 e1165.

Gerbaulet, A., Wickenhauser, C., Scholten, J., Peschke, K., Drube, S., Horny, H.P., Kamradt, T., Naumann, R., Muller, W., Krieg, T., *et al.* (2011). Mast cell hyperplasia, B-cell malignancy, and intestinal inflammation in mice with conditional expression of a constitutively active kit. Blood 117(6), 2012-2021.

Gleixner, K.V., Mayerhofer, M., Aichberger, K.J., Derdak, S., Sonneck, K., Bohm, A., Gruze, A., Samorapoompichit, P., Manley, P.W., Fabbro, D., *et al.* (2006). PKC412 inhibits in vitro growth of neoplastic human mast cells expressing the D816V-mutated variant of KIT: comparison with AMN107, imatinib, and cladribine (2CdA) and evaluation of cooperative drug effects. Blood 107(2), 752-759.

Goardon, N., Marchi, E., Atzberger, A., Quek, L., Schuh, A., Soneji, S., Woll, P., Mead, A., Alford, K.A., Rout, R., *et al.* (2011). Coexistence of LMPP-like and GMP-like leukemia stem cells in acute myeloid leukemia. Cancer Cell 19(1), 138-152.

Görgens, A., Radtke, S., Mollmann, M., Cross, M., Durig, J., Horn, P.A., and Giebel, B. (2013). Revision of the human hematopoietic tree: granulocyte subtypes derive from distinct hematopoietic lineages. Cell Rep 3(5), 1539-1552.

Gotlib, J., Kluin-Nelemans, H.C., George, T.I., Akin, C., Sotlar, K., Hermine, O., Awan, F.T., Hexner, E., Mauro, M.J., Sternberg, D.W., *et al.* (2016). Efficacy and Safety of Midostaurin in Advanced Systemic Mastocytosis. N Engl J Med 374(26), 2530-2541.

Greiner, G., Witzeneder, N., Berger, A., Schmetterer, K., Eisenwort, G., Schiefer, A.I., Roos, S., Popow-Kraupp, T., Mullauer, L., Zuber, J., *et al.* (2017). CCL2 is a KIT D816V-dependent modulator of the bone marrow microenvironment in systemic mastocytosis. Blood 129(3), 371-382.

Grootens, J., Ungerstedt, J.S., Nilsson, G., and Dahlin, J.S. (2018). Deciphering the differentiation trajectory from hematopoietic stem cells to mast cells. Blood Adv 2(17), 2273-2281.

Gulen, T., Hagglund, H., Dahlen, B., and Nilsson, G. (2014). High prevalence of anaphylaxis in patients with systemic mastocytosis - a single-centre experience. Clin Exp Allergy 44(1), 121-129.

Gülen, T., Hägglund, H., Dahlen, B., and Nilsson, G. (2016). Mastocytosis: the puzzling clinical spectrum and challenging diagnostic aspects of an enigmatic disease. J Intern Med 279(3), 211-228.

Gurish, M.F., Tao, H., Abonia, J.P., Arya, A., Friend, D.S., Parker, C.M., and Austen, K.F. (2001). Intestinal mast cell progenitors require CD49dbeta7 (alpha4beta7 integrin) for tissue-specific homing. J Exp Med 194(9), 1243-1252.

Hanssens, K., Brenet, F., Agopian, J., Georgin-Lavialle, S., Damaj, G., Cabaret, L., Chandesris, M.O., de Sepulveda, P., Hermine, O., Dubreuil, P., *et al.* (2014). SRSF2-p95 hotspot mutation is highly associated with advanced forms of mastocytosis and mutations in epigenetic regulator genes. Haematologica 99(5), 830-835.

Harir, N., Boudot, C., Friedbichler, K., Sonneck, K., Kondo, R., Martin-Lanneree, S., Kenner, L., Kerenyi, M., Yahiaoui, S., Gouilleux-Gruart, V., *et al.* (2008). Oncogenic Kit controls neoplastic mast cell growth through a Stat5/PI3-kinase signaling cascade. Blood 112(6), 2463-2473.

Hartmann, K., Escribano, L., Grattan, C., Brockow, K., Carter, M.C., Alvarez-Twose, I., Matito, A., Broesby-Olsen, S., Siebenhaar, F., Lange, M., *et al.* (2016). Cutaneous manifestations in patients with mastocytosis: Consensus report of the European Competence Network on Mastocytosis; the American Academy of Allergy, Asthma & Immunology; and the European Academy of Allergology and Clinical Immunology. J Allergy Clin Immunol 137(1), 35-45.

Hartmann, K., Wardelmann, E., Ma, Y., Merkelbach-Bruse, S., Preussner, L.M., Woolery, C., Baldus, S.E., Heinicke, T., Thiele, J., Buettner, R., *et al.* (2005). Novel germline mutation of KIT associated with familial gastrointestinal stromal tumors and mastocytosis. Gastroenterology 129(3), 1042-1046.

Harvima, I.T., Naukkarinen, A., Harvima, R.J., and Fraki, J.E. (1988). Immunoperoxidase and enzyme-histochemical demonstration of human skin tryptase in cutaneous mast cells in normal and mastocytoma skin. Arch Dermatol Res 280(6), 363-370.

Hauswirth, A.W., Escribano, L., Prados, A., Nunez, R., Mirkina, I., Kneidinger, M., Florian, S., Sonneck, K., Vales, A., Schernthaner, G.H., *et al.* (2008). CD203c is overexpressed on neoplastic mast cells in systemic mastocytosis and is upregulated upon IgE receptor cross-linking. Int J Immunopathol Pharmacol 21(4), 797-806.

Hoermann, G., Blatt, K., Greiner, G., Putz, E.M., Berger, A., Herrmann, H., Cerny-Reiterer, S., Gleixner, K.V., Walz, C., Hoetzenecker, K., *et al.* (2014a). CD52 is a molecular target in advanced systemic mastocytosis. FASEB J 28(8), 3540-3551.

Hoermann, G., Gleixner, K.V., Dinu, G.E., Kundi, M., Greiner, G., Wimazal, F., Hadzijusufovic, E., Mitterbauer, G., Mannhalter, C., Valent, P., *et al.* (2014b). The KIT D816V allele burden predicts survival in patients with mastocytosis and correlates with the WHO type of the disease. Allergy 69(6), 810-813.

Irani, A.M., Nilsson, G., Miettinen, U., Craig, S.S., Ashman, L.K., Ishizaka, T., Zsebo, K.M., and Schwartz, L.B. (1992). Recombinant human stem cell factor stimulates differentiation of mast cells from dispersed human fetal liver cells. Blood 80(12), 3009-3021.

Jabbar, K.J., Medeiros, L.J., Wang, S.A., Miranda, R.N., Johnson, M.R., Verstovsek, S., and Jorgensen, J.L. (2014). Flow cytometric immunophenotypic analysis of systemic mastocytosis involving bone marrow. Arch Pathol Lab Med 138(9), 1210-1214.

Jamur, M.C., Grodzki, A.C., Berenstein, E.H., Hamawy, M.M., Siraganian, R.P., and Oliver, C. (2005). Identification and characterization of undifferentiated mast cells in mouse bone marrow. Blood 105(11), 4282-4289.

Jara-Acevedo, M., Teodosio, C., Sanchez-Munoz, L., Alvarez-Twose, I., Mayado, A., Caldas, C., Matito, A., Morgado, J.M., Munoz-Gonzalez, J.I., Escribano, L., *et al.* (2015). Detection of the KIT D816V mutation in peripheral blood of systemic mastocytosis: diagnostic implications. Mod Pathol 28(8), 1138-1149.

Jawhar, M., Schwaab, J., Meggendorfer, M., Naumann, N., Horny, H.P., Sotlar, K., Haferlach, T., Schmitt, K., Fabarius, A., Valent, P., *et al.* (2017). The clinical and molecular diversity of mast cell leukemia with or without associated hematologic neoplasm. Haematologica 102(6), 1035-1043.

Jawhar, M., Schwaab, J., Schnittger, S., Meggendorfer, M., Pfirrmann, M., Sotlar, K., Horny, H.P., Metzgeroth, G., Kluger, S., Naumann, N., *et al.* (2016). Additional mutations in SRSF2, ASXL1 and/or RUNX1 identify a high-risk group of patients with KIT D816V(+) advanced systemic mastocytosis. Leukemia 30(1), 136-143.

Jawhar, M., Schwaab, J., Schnittger, S., Sotlar, K., Horny, H.P., Metzgeroth, G., Muller, N., Schneider, S., Naumann, N., Walz, C., *et al.* (2015). Molecular profiling of myeloid progenitor cells in multi-mutated advanced systemic mastocytosis identifies KIT D816V as a distinct and late event. Leukemia 29(5), 1115-1122.

Jensen, B., Broesby-Olsen, S., Bindslev-Jensen, C., and Nielsen, D.S. (2019). Everyday life and mastocytosis from a patient perspective-a qualitative study. Journal of clinical nursing 28(7-8), 1114-1124.

Kanakura, Y., Kuriu, A., Waki, N., Nakano, T., Asai, H., Yonezawa, T., and Kitamura, Y. (1988). Changes in numbers and types of mast cell colony-forming cells in the peritoneal cavity of mice after injection of distilled water: evidence that mast cells suppress differentiation of bone marrow-derived precursors. Blood 71(3), 573-580.

Kasamon, Y.L., Ko, C.W., Subramaniam, S., Ma, L., Yang, Y., Nie, L., Shord, S., Przepiorka, D., Farrell, A.T., McKee, A.E., *et al.* (2018). FDA Approval Summary: Midostaurin for the Treatment of Advanced Systemic Mastocytosis. Oncologist 23(12), 1511-1519.

Kempuraj, D., Saito, H., Kaneko, A., Fukagawa, K., Nakayama, M., Toru, H., Tomikawa, M., Tachimoto, H., Ebisawa, M., Akasawa, A., *et al.* (1999). Characterization of mast cell-committed progenitors present in human umbilical cord blood. Blood 93(10), 3338-3346.

Kinoshita, T., Sawai, N., Hidaka, E., Yamashita, T., and Koike, K. (1999). Interleukin-6 Directly Modulates Stem Cell Factor-Dependent Development of Human Mast Cells Derived From CD34⁺Cord Blood Cells. Blood 94(2), 496-508.

Kirshenbaum, A.S., Goff, J.P., Kessler, S.W., Mican, J.M., Zsebo, K.M., and Metcalfe, D.D. (1992). Effect of IL-3 and stem cell factor on the appearance of human basophils and mast cells from CD34+ pluripotent progenitor cells. J Immunol 148(3), 772-777.

Kirshenbaum, A.S., Goff, J.P., Semere, T., Foster, B., Scott, L.M., and Metcalfe, D.D. (1999). Demonstration that human mast cells arise from a progenitor cell population that is CD34(+), c-kit(+), and expresses aminopeptidase N (CD13). Blood 94(7), 2333-2342.

Kirshenbaum, A.S., Kessler, S.W., Goff, J.P., and Metcalfe, D.D. (1991). Demonstration of the origin of human mast cells from CD34+ bone marrow progenitor cells. J Immunol 146(5), 1410-1415.

Kitamura, Y., Shimada, M., Hatanaka, K., and Miyano, Y. (1977). Development of mast cells from grafted bone marrow cells in irradiated mice. Nature 268(5619), 442-443.

Knol, E.F., Mul, F.P., Jansen, H., Calafat, J., and Roos, D. (1991). Monitoring human basophil activation via CD63 monoclonal antibody 435. J Allergy Clin Immunol 88(3 Pt 1), 328-338.

Kocabas, C.N., Yavuz, A.S., Lipsky, P.E., Metcalfe, D.D., and Akin, C. (2005). Analysis of the lineage relationship between mast cells and basophils using the c-kit D816V mutation as a biologic signature. J Allergy Clin Immunol 115(6), 1155-1161.

Krauth, M.T., Bohm, A., Agis, H., Sonneck, K., Samorapoompichit, P., Florian, S., Sotlar, K., and Valent, P. (2007). Effects of the CD33-targeted drug gemtuzumab ozogamicin (Mylotarg) on growth and mediator secretion in human mast cells and blood basophils. Exp Hematol 35(1), 108-116.

Kristensen, T., Broesby-Olsen, S., Vestergaard, H., Bindslev-Jensen, C., and Moller, M.B. (2013). KIT D816V mutation-positive cell fractions in lesional skin biopsies from adults with systemic mastocytosis. Dermatology (Basel, Switzerland) 226(3), 233-237.

Kristensen, T., Preiss, B., Broesby-Olsen, S., Vestergaard, H., Friis, L., and Moller, M.B. (2012). Systemic mastocytosis is uncommon in KIT D816V mutation positive core-binding factor acute myeloid leukemia. Leuk Lymphoma 53(7), 1338-1344.

Kristensen, T., Vestergaard, H., and Moller, M.B. (2011). Improved detection of the KIT D816V mutation in patients with systemic mastocytosis using a quantitative and highly sensitive real-time qPCR assay. J Mol Diagn 13(2), 180-188.

Laine, E., Chauvot de Beauchene, I., Perahia, D., Auclair, C., and Tchertanov, L. (2011). Mutation D816V alters the internal structure and dynamics of c-KIT receptor cytoplasmic region: implications for dimerization and activation mechanisms. PLoS Comput Biol 7(6), e1002068.

Lennartsson, J., and Ronnstrand, L. (2012). Stem cell factor receptor/c-Kit: from basic science to clinical implications. Physiol Rev 92(4), 1619-1649.

Letard, S., Yang, Y., Hanssens, K., Palmerini, F., Leventhal, P.S., Guery, S., Moussy, A., Kinet, J.P., Hermine, O., and Dubreuil, P. (2008). Gain-of-function mutations in the extracellular domain of KIT are common in canine mast cell tumors. Mol Cancer Res 6(7), 1137-1145.

Li, Z., Liu, S., Xu, J., Zhang, X., Han, D., Liu, J., Xia, M., Yi, L., Shen, Q., Xu, S., *et al.* (2018). Adult Connective Tissue-Resident Mast Cells Originate from Late Erythro-Myeloid Progenitors. Immunity 49(4), 640-653 e645.

Lim, K.H., Tefferi, A., Lasho, T.L., Finke, C., Patnaik, M., Butterfield, J.H., McClure, R.F., Li, C.Y., and Pardanani, A. (2009). Systemic mastocytosis in 342 consecutive adults: survival studies and prognostic factors. Blood 113(23), 5727-5736.

Lotfi-Emran, S., Ward, B.R., Le, Q.T., Pozez, A.L., Manjili, M.H., Woodfolk, J.A., and Schwartz, L.B. (2018). Human mast cells present antigen to autologous CD4(+) T cells. J Allergy Clin Immunol 141(1), 311-321 e310.

Lubke, J., Naumann, N., Kluger, S., Schwaab, J., Metzgeroth, G., Evans, E., Gardino, A.K., Lengauer, C., Hofmann, W.K., Fabarius, A., *et al.* (2019). Inhibitory effects of midostaurin and avapritinib on myeloid progenitors derived from patients with KIT D816V positive advanced systemic mastocytosis. Leukemia.

Lyberg, K., Ali, H.A., Grootens, J., Kjellander, M., Tirfing, M., Arock, M., Hagglund, H., Nilsson, G., and Ungerstedt, J. (2017). Histone deacetylase inhibitor SAHA mediates mast cell death and epigenetic silencing of constitutively active D816V KIT in systemic mastocytosis. Oncotarget 8(6), 9647-9659.

Ma, Y.S., Zeng, S., Metcalfe, D.D., Akin, C., Dimitrijevic, S., Butterfield, J.H., McMahon, G., and Longley, B.J. (2002). The c-KIT mutation causing human mastocytosis is resistant to STI571 and other KIT kinase inhibitors; kinases with enzymatic site mutations show different inhibitor sensitivity profiles than wild-type kinases and those with regulatory-type mutations. Blood 99(5), 1741-1744.

Maaninka, K., Lappalainen, J., and Kovanen, P.T. (2013). Human mast cells arise from a common circulating progenitor. J Allergy Clin Immunol 132(2), 463-469 e463.

Majeti, R., Park, C.Y., and Weissman, I.L. (2007). Identification of a hierarchy of multipotent hematopoietic progenitors in human cord blood. Cell Stem Cell 1(6), 635-645.

Manz, M.G., Miyamoto, T., Akashi, K., and Weissman, I.L. (2002). Prospective isolation of human clonogenic common myeloid progenitors. Proceedings of the National Academy of Sciences of the United States of America 99(18), 11872-11877.

Matito, A., Morgado, J.M., Álvarez-Twose, I., Sánchez-Muñoz, L., Pedreira, C.E., Jara-Acevedo, M., Teodosio, C., Sánchez-López, P., Fernández-Núñez, E., Moreno-Borque, R., *et al.* (2013). Serum tryptase monitoring in indolent systemic mastocytosis: association with disease features and patient outcome. PLoS One 8(10), e76116.

Matsumoto, K., Appiah-Pippim, J., Schleimer, R.P., Bickel, C.A., Beck, L.A., and Bochner, B.S. (1998). CD44 and CD69 represent different types of cell-surface activation markers for human eosinophils. Am J Respir Cell Mol Biol 18(6), 860-866.

Mayado, A., Teodosio, C., Dasilva-Freire, N., Jara-Acevedo, M., Garcia-Montero, A.C., Alvarez-Twose, I., Sanchez-Munoz, L., Matito, A., Caldas, C., Munoz-Gonzalez, J.I., *et al.* (2018). Characterization of CD34(+) hematopoietic cells in systemic mastocytosis: Potential role in disease dissemination. Allergy 73(6), 1294-1304.

Mayado, A., Teodosio, C., Garcia-Montero, A.C., Matito, A., Rodriguez-Caballero, A., Morgado, J.M., Muniz, C., Jara-Acevedo, M., Alvarez-Twose, I., Sanchez-Munoz, L., *et al.* (2016). Increased IL6 plasma levels in indolent systemic mastocytosis patients are associated with high risk of disease progression. Leukemia 30(1), 124-130.

Méni, C., Bruneau, J., Georgin-Lavialle, S., Le Saché de Peufeilhoux, L., Damaj, G., Hadj-Rabia, S., Fraitag, S., Dubreuil, P., Hermine, O., and Bodemer, C. (2015). Paediatric mastocytosis: a systematic review of 1747 cases. British Journal of Dermatology 172(3), 642-651.

Mian, S.A., Smith, A.E., Kulasekararaj, A.G., Kizilors, A., Mohamedali, A.M., Lea, N.C., Mitsopoulos, K., Ford, K., Nasser, E., Seidl, T., *et al.* (2013). Spliceosome mutations exhibit specific associations with epigenetic modifiers and proto-oncogenes mutated in myelodysplastic syndrome. Haematologica 98(7), 1058-1066.

Mitsui, H., Furitsu, T., Dvorak, A.M., Irani, A.M., Schwartz, L.B., Inagaki, N., Takei, M., Ishizaka, K., Zsebo, K.M., Gillis, S., *et al.* (1993). Development of human mast cells from umbilical cord blood cells by recombinant human and

murine c-kit ligand. Proceedings of the National Academy of Sciences of the United States of America 90(2), 735-739.

Moller, C., Alfredsson, J., Engstrom, M., Wootz, H., Xiang, Z., Lennartsson, J., Jonsson, J.I., and Nilsson, G. (2005). Stem cell factor promotes mast cell survival via inactivation of FOXO3a-mediated transcriptional induction and MEK-regulated phosphorylation of the proapoptotic protein Bim. Blood 106(4), 1330-1336.

Montagner, S., Leoni, C., Emming, S., Della Chiara, G., Balestrieri, C., Barozzi, I., Piccolo, V., Togher, S., Ko, M., Rao, A., *et al.* (2016). TET2 Regulates Mast Cell Differentiation and Proliferation through Catalytic and Non-catalytic Activities. Cell Rep 15(7), 1566-1579.

Moon, T.C., Befus, A.D., and Kulka, M. (2014). Mast cell mediators: their differential release and the secretory pathways involved. Front Immunol 5569.

Morgado, J.M., Sanchez-Munoz, L., Teodosio, C.G., Jara-Acevedo, M., Alvarez-Twose, I., Matito, A., Fernandez-Nunez, E., Garcia-Montero, A., Orfao, A., and Escribano, L. (2012). Immunophenotyping in systemic mastocytosis diagnosis: 'CD25 positive' alone is more informative than the 'CD25 and/or CD2' WHO criterion. Mod Pathol 25(4), 516-521.

Mori, Y., Iwasaki, H., Kohno, K., Yoshimoto, G., Kikushige, Y., Okeda, A., Uike, N., Niiro, H., Takenaka, K., Nagafuji, K., *et al.* (2009). Identification of the human eosinophil lineage-committed progenitor: revision of phenotypic definition of the human common myeloid progenitor. J Exp Med 206(1), 183-193.

Morrison, S.J., and Scadden, D.T. (2014). The bone marrow niche for haematopoietic stem cells. Nature 505(7483), 327-334.

Motakis, E., Guhl, S., Ishizu, Y., Itoh, M., Kawaji, H., de Hoon, M., Lassmann, T., Carninci, P., Hayashizaki, Y., Zuberbier, T., *et al.* (2014). Redefinition of the human mast cell transcriptome by deep-CAGE sequencing. Blood 123(17), e58-67.

Mueller, N., Wicklein, D., Eisenwort, G., Jawhar, M., Berger, D., Stefanzl, G., Greiner, G., Boehm, A., Kornauth, C., Muellauer, L., *et al.* (2018). CD44 is a RAS/STAT5-regulated invasion receptor that triggers disease expansion in advanced mastocytosis. Blood 132(18), 1936-1950.

Nemeth, K., Wilson, T.M., Ren, J.J., Sabatino, M., Stroncek, D.M., Krepuska, M., Bai, Y., Robey, P.G., Metcalfe, D.D., and Mezey, E. (2015). Impaired function of bone marrow stromal cells in systemic mastocytosis. Stem Cell Res 15(1), 42-53.

Nilsson, G., Butterfield, J.H., Nilsson, K., and Siegbahn, A. (1994a). Stem cell factor is a chemotactic factor for human mast cells. J Immunol 153(8), 3717-3723.

Nilsson, G., Miettinen, U., Ishizaka, T., Ashman, L.K., Irani, A.M., and Schwartz, L.B. (1994b). Interleukin-4 inhibits the expression of Kit and tryptase during stem cell factor-dependent development of human mast cells from fetal liver cells. Blood 84(5), 1519-1527.

Notta, F., Doulatov, S., Laurenti, E., Poeppl, A., Jurisica, I., and Dick, J.E. (2011). Isolation of Single Human Hematopoietic Stem Cells Capable of Long-Term Multilineage Engraftment. Science 333(6039), 218-221.

Notta, F., Zandi, S., Takayama, N., Dobson, S., Gan, O.I., Wilson, G., Kaufmann, K.B., McLeod, J., Laurenti, E., Dunant, C.F., *et al.* (2016). Distinct routes of lineage development reshape the human blood hierarchy across ontogeny. Science 351(6269), aab2116.

Nunez-Lopez, R., Escribano, L., Schernthaner, G.H., Prados, A., Rodriguez-Gonzalez, R., Diaz-Agustin, B., Lopez, A., Hauswirth, A., Valent, P., Almeida, J., *et al.* (2003). Overexpression of complement receptors and related antigens on the surface of bone marrow mast cells in patients with systemic mastocytosis. Br J Haematol 120(2), 257-265.

Ocmant, A., Peignois, Y., Mulier, S., Hanssens, L., Michils, A., and Schandene, L. (2007). Flow cytometry for basophil activation markers: the measurement of CD203c up-regulation is as reliable as CD63 expression in the diagnosis of cat allergy. Journal of immunological methods 320(1-2), 40-48.

Ogawa, S. (2019). Genetics of MDS. Blood 133(10), 1049-1059.

Pardanani, A., Lasho, T., Elala, Y., Wassie, E., Finke, C., Reichard, K.K., Chen, D., Hanson, C.A., Ketterling, R.P., and Tefferi, A. (2016a). Next-generation sequencing in systemic mastocytosis: Derivation of a mutation-augmented clinical prognostic model for survival. Am J Hematol 91(9), 888-893.

Pardanani, A., Reeder, T., Li, C.Y., and Tefferi, A. (2003). Eosinophils are derived from the neoplastic clone in patients with systemic mastocytosis and eosinophilia. Leuk Res 27(10), 883-885.

Pardanani, A., Reichard, K.K., Zblewski, D., Abdelrahman, R.A., Wassie, E.A., Morice Ii, W.G., Brooks, C., Grogg, K.L., Hanson, C.A., Tefferi, A., *et al.* (2016b). CD123 immunostaining patterns in systemic mastocytosis: differential expression in disease subgroups and potential prognostic value. Leukemia 30(4), 914-918.

Parikh, S.A., Kantarjian, H.M., Richie, M.A., Cortes, J.E., and Verstovsek, S. (2010). Experience with everolimus (RAD001), an oral mammalian target of rapamycin inhibitor, in patients with systemic mastocytosis. Leuk Lymphoma 51(2), 269-274.

Paul, F., Arkin, Y., Giladi, A., Jaitin, D.A., Kenigsberg, E., Keren-Shaul, H., Winter, D., Lara-Astiaso, D., Gury, M., Weiner, A., *et al.* (2015). Transcriptional Heterogeneity and Lineage Commitment in Myeloid Progenitors. Cell 163(7), 1663-1677.

Peter, B., Bibi, S., Eisenwort, G., Wingelhofer, B., Berger, D., Stefanzl, G., Blatt, K., Herrmann, H., Hadzijusufovic, E., Hoermann, G., *et al.* (2018). Drug-induced inhibition of phosphorylation of STAT5 overrides drug resistance in neoplastic mast cells. Leukemia 32(4), 1016-1022.

Rabenhorst, A., Leja, S., Schwaab, J., Gehring, M., Forster, A., Arock, M., Reiter, A., Raap, U., and Hartmann, K. (2016). Expression of programmed cell death ligand-1 in mastocytosis correlates with disease severity. J Allergy Clin Immunol 137(1), 314-318 e315.

Ryan, R.J., Akin, C., Castells, M., Wills, M., Selig, M.K., Nielsen, G.P., Ferry, J.A., and Hornick, J.L. (2013). Mast cell sarcoma: a rare and potentially under-recognized diagnostic entity with specific therapeutic implications. Mod Pathol 26(4), 533-543.

Saito, H., Ebisawa, M., Tachimoto, H., Shichijo, M., Fukagawa, K., Matsumoto, K., Iikura, Y., Awaji, T., Tsujimoto, G., Yanagida, M., *et al.* (1996). Selective growth of human mast cells induced by Steel factor, IL-6, and prostaglandin E2 from cord blood mononuclear cells. J Immunol 157(1), 343-350.

Saleh, R., Wedeh, G., Herrmann, H., Bibi, S., Cerny-Reiterer, S., Sadovnik, I., Blatt, K., Hadzijusufovic, E., Jeanningros, S., Blanc, C., *et al.* (2014). A new human mast cell line expressing a functional IgE receptor converts to tumorigenic growth by KIT D816V transfection. Blood 124(1), 111-120.

Sano, K., Yamauchi, K., Hoshi, H., Honma, M., Tamura, G., and Shirato, K. (1998). CD44 on blood eosinophils as a novel marker of bronchial asthma management. Allergology International 47(1), 29-35.

Schernthaner, G.H., Hauswirth, A.W., Baghestanian, M., Agis, H., Ghannadan, M., Worda, C., Krauth, M.T., Printz, D., Fritsch, G., Sperr, W.R., *et al.* (2005). Detection of differentiation- and activation-linked cell surface antigens on cultured mast cell progenitors. Allergy 60(10), 1248-1255.

Schlessinger, J. (2000). Cell Signaling by Receptor Tyrosine Kinases. Cell 103(2), 211-225.

Schwaab, J., Schnittger, S., Sotlar, K., Walz, C., Fabarius, A., Pfirrmann, M., Kohlmann, A., Grossmann, V., Meggendorfer, M., Horny, H.P., *et al.* (2013). Comprehensive mutational profiling in advanced systemic mastocytosis. Blood 122(14), 2460-2466.

Schwartz, L.B., Sakai, K., Bradford, T.R., Ren, S., Zweiman, B., Worobec, A.S., and Metcalfe, D.D. (1995). The alpha form of human tryptase is the predominant type present in blood at baseline in normal subjects and is elevated in those with systemic mastocytosis. The Journal of clinical investigation 96(6), 2702-2710.

Shultz, L.D., Lyons, B.L., Burzenski, L.M., Gott, B., Chen, X., Chaleff, S., Kotb, M., Gillies, S.D., King, M., Mangada, J., *et al.* (2005). Human lymphoid and myeloid cell development in NOD/LtSz-scid IL2R gamma null mice engrafted with mobilized human hemopoietic stem cells. J Immunol 174(10), 6477-6489.

Sirvent, A.E., Gonzalez, C., Enriquez, R., Fernandez, J., Millan, I., Barber, X., and Amoros, F. (2010). Serum tryptase levels and markers of renal dysfunction in a population with chronic kidney disease. Journal of nephrology 23(3), 282-290.

Sonoda, T., Hayashi, C., and Kitamura, Y. (1983). Presence of mast cell precursors in the yolk sac of mice. Dev Biol 97(1), 89-94.

Sotlar, K., Bache, A., Stellmacher, F., Bultmann, B., Valent, P., and Horny, H.P. (2008). Systemic mastocytosis associated with chronic idiopathic myelofibrosis: a distinct subtype of systemic mastocytosis associated with a [corrected] clonal hematological non-mast [corrected] cell lineage disorder carrying the activating point mutations KITD816V and JAK2V617F. J Mol Diagn 10(1), 58-66.

Sotlar, K., Cerny-Reiterer, S., Petat-Dutter, K., Hessel, H., Berezowska, S., Mullauer, L., Valent, P., and Horny, H.P. (2011). Aberrant expression of CD30 in neoplastic mast cells in high-grade mastocytosis. Mod Pathol 24(4), 585-595.

Soucie, E., Hanssens, K., Mercher, T., Georgin-Lavialle, S., Damaj, G., Livideanu, C., Chandesris, M.O., Acin, Y., Letard, S., de Sepulveda, P., *et al.* (2012). In aggressive forms of mastocytosis, TET2 loss cooperates with c-KITD816V to transform mast cells. Blood 120(24), 4846-4849.

Spangrude, G.J., Heimfeld, S., and Weissman, I.L. (1988). Purification and characterization of mouse hematopoietic stem cells. Science 241(4861), 58-62.

Sperr, W.R., Jordan, J.H., Fiegl, M., Escribano, L., Bellas, C., Dirnhofer, S., Semper, H., Simonitsch-Klupp, I., Horny, H.P., and Valent, P. (2002). Serum tryptase levels in patients with mastocytosis: correlation with mast cell burden and implication for defining the category of disease. International archives of allergy and immunology 128(2), 136-141.

Sperr, W.R., Pfeiffer, T., Hoermann, G., Herndlhofer, S., Sillaber, C., Mannhalter, C., Kundi, M., and Valent, P. (2014). Serum-tryptase at diagnosis: a novel biomarker improving prognostication in Ph+ CML. Am J Cancer Res 5(1), 354-362.

Sundstrom, M., Vliagoftis, H., Karlberg, P., Butterfield, J.H., Nilsson, K., Metcalfe, D.D., and Nilsson, G. (2003). Functional and phenotypic studies of two variants of a human mast cell line with a distinct set of mutations in the c-kit proto-oncogene. Immunology 108(1), 89-97.

Sutherland, H.J., Eaves, C.J., Eaves, A.C., Dragowska, W., and Lansdorp, P.M. (1989). Characterization and partial purification of human marrow cells capable of initiating long-term hematopoiesis in vitro. Blood 74(5), 1563-1570.

Tefferi, A., Shah, S., Reichard, K.K., Hanson, C.A., and Pardanani, A. (2019). Smoldering mastocytosis: Survival comparisons with indolent and aggressive mastocytosis. Am J Hematol 94(1), E1-E2.

Teodosio, C., Garcia-Montero, A.C., Jara-Acevedo, M., Sanchez-Munoz, L., Alvarez-Twose, I., Nunez, R., Schwartz, L.B., Walls, A.F., Escribano, L., and Orfao, A. (2010). Mast cells from different molecular and prognostic subtypes of systemic mastocytosis display distinct immunophenotypes. J Allergy Clin Immunol 125(3), 719-726.

Teodosio, C., Garcia-Montero, A.C., Jara-Acevedo, M., Sanchez-Munoz, L., Pedreira, C.E., Alvarez-Twose, I., Matarraz, S., Morgado, J.M., Barcena, P., Matito, A., *et al.* (2013). Gene expression profile of highly purified bone marrow mast cells in systemic mastocytosis. J Allergy Clin Immunol 131(4), 1213-1224.

Teodosio, C., Mayado, A., Sanchez-Munoz, L., Morgado, J.M., Jara-Acevedo, M., Alvarez-Twose, I., Garcia-Montero, A.C., Matito, A., Caldas, C., Escribano, L., *et al.* (2015). The immunophenotype of mast cells and its utility in the diagnostic work-up of systemic mastocytosis. J Leukoc Biol 97(1), 49-59.

Theoharides, T.C., Boucher, W., and Spear, K. (2002). Serum interleukin-6 reflects disease severity and osteoporosis in mastocytosis patients. International archives of allergy and immunology 128(4), 344-350.

Tobío, A., Bandara, G., Morris, D.A., Kim, D.K., O'Connell, M.P., Komarow, H.D., Carter, M.C., Smrz, D., Metcalfe, D.D., and Olivera, A. (2019). Oncogenic D816V-KIT signaling in mast cells causes persistent IL-6 production. Haematologicahaematol.2018.212126.

Tsai, F.Y., and Orkin, S.H. (1997). Transcription factor GATA-2 is required for proliferation/survival of early hematopoietic cells and mast cell formation, but not for erythroid and myeloid terminal differentiation. Blood 89(10), 3636-3643.

Tusi, B.K., Wolock, S.L., Weinreb, C., Hwang, Y., Hidalgo, D., Zilionis, R., Waisman, A., Huh, J.R., Klein, A.M., and Socolovsky, M. (2018). Population snapshots predict early haematopoietic and erythroid hierarchies. Nature 555(7694), 54-60.

Ustun, C., Reiter, A., Scott, B.L., Nakamura, R., Damaj, G., Kreil, S., Shanley, R., Hogan, W.J., Perales, M.A., Shore, T., *et al.* (2014). Hematopoietic stem-cell transplantation for advanced systemic mastocytosis. J Clin Oncol 32(29), 3264-3274.

Valent, P., Akin, C., and Metcalfe, D.D. (2017). Mastocytosis: 2016 updated WHO classification and novel emerging treatment concepts. Blood 129(11), 1420-1427.

Valent, P., Horny, H.P., and Arock, M. (2018). The underestimated role of basophils in Ph(+) chronic myeloid leukaemia. Eur J Clin Invest 48(10), e13000.

Valent, P., Schmidt, G., Besemer, J., Mayer, P., Zenke, G., Liehl, E., Hinterberger, W., Lechner, K., Maurer, D., and Bettelheim, P. (1989). Interleukin-3 is a differentiation factor for human basophils. Blood 73(7), 1763-1769.

Valent, P., Spanblochl, E., Sperr, W.R., Sillaber, C., Zsebo, K.M., Agis, H., Strobl, H., Geissler, K., Bettelheim, P., and Lechner, K. (1992). Induction of differentiation of human mast cells from bone marrow and peripheral blood mononuclear cells by recombinant human stem cell factor/kit-ligand in long-term culture. Blood 80(9), 2237-2245.

Valent, P., Sperr, W.R., Sotlar, K., Reiter, A., Akin, C., Gotlib, J., Horny, H.P., and Arock, M. (2014). The serum tryptase test: an emerging robust biomarker in clinical hematology. Expert Rev Hematol 7(5), 683-690.

van Doormaal, J.J., Arends, S., Brunekreeft, K.L., van der Wal, V.B., Sietsma, J., van Voorst Vader, P.C., Oude Elberink, J.N., Kluin-Nelemans, J.C., van der Veer, E., and de Monchy, J.G. (2013). Prevalence of indolent systemic mastocytosis in a Dutch region. J Allergy Clin Immunol 131(5), 1429-1431 e1421.

Velten, L., Haas, S.F., Raffel, S., Blaszkiewicz, S., Islam, S., Hennig, B.P., Hirche, C., Lutz, C., Buss, E.C., Nowak, D., *et al.* (2017). Human haematopoietic stem cell lineage commitment is a continuous process. Nat Cell Biol 19(4), 271-281.

Webster, J.D., Kiupel, M., and Yuzbasiyan-Gurkan, V. (2006). Evaluation of the kinase domain of c-KIT in canine cutaneous mast cell tumors. BMC Cancer 685.

Weidemann, R.R., Behrendt, R., Schoedel, K.B., Muller, W., Roers, A., and Gerbaulet, A. (2017). Constitutive Kit activity triggers B-cell acute lymphoblastic leukemia-like disease in mice. Exp Hematol 4545-55 e46.

Xia, H.Z., Du, Z., Craig, S., Klisch, G., Noben-Trauth, N., Kochan, J.P., Huff, T.H., Irani, A.M., and Schwartz, L.B. (1997). Effect of recombinant human IL-4 on tryptase, chymase, and Fc epsilon receptor type I expression in recombinant human stem cell factor-dependent fetal liver-derived human mast cells. J Immunol 159(6), 2911-2921.

Xiang, Z., Block, M., Lofman, C., and Nilsson, G. (2001). IgE-mediated mast cell degranulation and recovery monitored by time-lapse photography. J Allergy Clin Immunol 108(1), 116-121.

Xiang, Z., Kreisel, F., Cain, J., Colson, A., and Tomasson, M.H. (2007). Neoplasia driven by mutant c-KIT is mediated by intracellular, not plasma membrane, receptor signaling. Mol Cell Biol 27(1), 267-282.

Yacoub, A., and Prochaska, L. (2016). Ruxolitinib improves symptoms and quality of life in a patient with systemic mastocytosis. Biomark Res 42.

Yavuz, A.S., Lipsky, P.E., Yavuz, S., Metcalfe, D.D., and Akin, C. (2002). Evidence for the involvement of a hematopoietic progenitor cell in systemic mastocytosis from single-cell analysis of mutations in the c-kit gene. Blood 100(2), 661-665.

Yoshimura, C., Yamaguchi, M., Iikura, M., Izumi, S., Kudo, K., Nagase, H., Ishii, A., Walls, A.F., Ra, C., Iwata, T., *et al.* (2002). Activation markers of human basophils: CD69 expression is strongly and preferentially induced by IL-3. Journal of Allergy and Clinical Immunology 109(5), 817-823.

Zappulla, J.P., Dubreuil, P., Desbois, S., Letard, S., Hamouda, N.B., Daeron, M., Delsol, G., Arock, M., and Liblau, R.S. (2005). Mastocytosis in mice expressing human Kit receptor with the activating Asp816Val mutation. J Exp Med 202(12), 1635-1641.

Zheng, S., Papalexi, E., Butler, A., Stephenson, W., and Satija, R. (2018). Molecular transitions in early progenitors during human cord blood hematopoiesis. Mol Syst Biol 14(3), e8041.

Zheng, X., Li, A.S., Zheng, H., Zhao, D., Guan, D., and Zou, H. (2015). Different associations of CD45 isoforms with STAT3, PKC and ERK regulate IL-6-induced proliferation in myeloma. PLoS One 10(3), e0119780.

Zon, L.I. (2008). Intrinsic and extrinsic control of haematopoietic stem-cell self-renewal. Nature 453(7193), 306-313.